

The Clinical Physiology of Water Metabolism

Part I: The Physiologic Regulation of Arginine Vasopressin Secretion and Thirst

RICHARD E. WEITZMAN, MD, *Torrance, California*, and
CHARLES R. KLEEMAN, MD, *Los Angeles*

Water balance is tightly regulated within a tolerance of less than 1 percent by a physiologic control system located in the hypothalamus. Body water homeostasis is achieved by balancing renal and nonrenal water losses with appropriate water intake. The major stimulus to thirst is increased osmolality of body fluids as perceived by osmoreceptors in the anteroventral hypothalamus. Hypovolemia also has an important effect on thirst which is mediated by arterial baroreceptors and by the renin-angiotensin system. Renal water loss is determined by the circulating level of the antidiuretic hormone, arginine vasopressin (AVP). AVP is synthesized in specialized neurosecretory cells located in the supraoptic and paraventricular nuclei in the hypothalamus and is transported in neurosecretory granules down elongated axons to the posterior pituitary. Depolarization of the neurosecretory neurons results in the exocytosis of the granules and the release of AVP and its carrier protein (neurophysin) into the circulation. AVP is secreted in response to a wide variety of stimuli. Change in body fluid osmolality is the most potent factor affecting AVP secretion, but hypovolemia, the renin-angiotensin system, hypoxia, hypercapnia, hyperthermia and pain also have important effects. Many drugs have been shown to stimulate the release of AVP as well. Small changes in plasma AVP concentration of from 0.5 to 4 μ U per ml have major effects on urine osmolality and renal water handling.

Dr. Weitzman is Assistant Professor of Medicine, UCLA School of Medicine; Division of Nephrology and Hypertension, Harbor-UCLA Medical Center, Torrance, CA; and Dr. Kleeman is Factor Family Foundation Professor of Nephrology and Medicine, Emeritus Chief, Division of Nephrology, Department of Medicine, Director, Center for Health Enhancement, Education and Research, UCLA Center for the Health Sciences, Los Angeles.

Part I of a three-part article. Parts II and III will be published in subsequent issues.

Supported by a grant from the Greater Los Angeles Affiliate of the American Heart Association, and the James Fleming Fund.

Reprint requests to: Charles R. Kleeman, MD, Director, Center for Health Enhancement, Education and Research, Department of Medicine, UCLA Center for the Health Sciences, Los Angeles, CA 90024.

General Concepts of Water Metabolism

IN NORMAL HUMANS, osmolality, or the concentration of osmotically active solutes in body fluids, is remarkably constant despite large variations in water and solute intake and excretion. Every kilogram of body water contains 285 to 290 mOsm of solute, consisting primarily of salts of sodium in extracellular fluid and of potassium in intracellular fluid. The identical osmolality of intracellular and extracellular fluids is produced by free movement of water across all cellular and subcellular membranes, governed only by the physical forces of osmosis and diffusion. A gain or loss of free water will be shared by all major body compartments (vascular, interstitial and intracellular) in proportion to their relative sizes. The single exception to this free movement is the control of water permeability of the distal portion of the mammalian nephron by arginine vasopressin (AVP), antidiuretic hormone. Among all vertebrates, only mammals (and to some extent, birds) possess the unique ability to excrete a hyperosmotic urine and thus conserve free water when the supply of available water is limited. It has been suggested that this ability of the mammalian kidney to concentrate fluids may have played an important role in our ultimate domination over the dinosaurs and other reptiles at the end of the Mesozoic era. There is a continuous decrease in the proportion of total body water (and particularly the extracellular compartment) with growth and aging, as well as a difference between men and women (Table 1).

The day-to-day fluctuations in total body water in a normal person are very small, amounting to approximately 0.2 percent of body weight per 24 hours. Although infants have a relative excess of body water and extracellular volume as related to total body weight, the surface area, oxygen consumption, cardiac output, insensible water loss, renal water excretion and overall metabolism are all high in relation to total body water. Therefore, when one compares these fundamental metabolic measurements with total body water, infants and young children are seen to be more vulnerable to water deficit and dehydration than adults.¹

The average normal intake and loss of water in a day by an adult human is given in Table 2. Even with maximal renal conservation of water, the body is unable to prevent a continuous loss of insensible fluid through the skin, lungs and gastro-

ABBREVIATIONS USED IN TEXT

AVP=arginine vasopressin
CSF=cerebrospinal fluid
U/P=urinary to plasma (ratio)

intestinal tract. For the replacement of this extrarenal loss, a person must rely on adequate water ingestion. Humans and other mammals vary considerably in their need for an exogenous source of water in their diet during maximal renal conservation. Of all mammals, only certain desert rodents can minimize their renal and extrarenal water losses sufficiently to maintain their water balance from that available in the preformed and oxidative water found in the desert plants they ingest. These rodents can concentrate their urine to a level four to five times greater than that of humans, and by living in underground burrows

TABLE 1.—*Body Water Compartments Throughout Life Span**

Age	Sex	Body Weight (Kg)	Extra-cellular Water (Percent)	Intra-cellular Water (Percent)	Total Body Water (Percent)
0-11 days ...			41.6	34.8	76.4
11-180 days ..			34.9	37.9	72.8
½-2 years ...			27.5	34.7	62.2
2-7 years ...			25.6	36.9	62.5
7-14 years ..			17.5	46.7	64.2
23-54 years ..	♂	72.5	23.4	30.9	54.3
			± 0.64	± 0.89	± 1.39†
23-51 years ..	♀	59.3	22.7	25.9	48.6
			± 0.54	± 0.96	± 1.47
71-84 years ..	♂	68.1	25.4	25.4	50.8
			± 1.36	± 0.58	± 1.55
61-74 years ..	♀	63.9	21.4	22.4	43.4
			± 0.45	± 0.97	± 1.32

*Modified from Parker HV, Olesen KH, McMurrey J, et al: *In Ciba Foundation, Colloquia on Ageing*, Vol 4. Boston, Little, Brown and Company, 1958.

†Standard error of the mean.

TABLE 2.—*Average Daily Intake and Output of Water in a Normal Adult Human**

Water Intake (ml)			Water Output (ml)		
Source	Oblig-atory	Facul-tative	Source	Oblig-atory	Facul-tative
Drink	650	1,000	Urine	700	1,000
Preformed ..	750	Skin	500
Oxidative ..	350	Lungs	400
			Feces	150
Subtotals ..	1,750	1,000	Subtotals ..	1,750	1,000
Total	2,750	Total	2,750

*From Wolf AV: *Thirst*. Springfield, IL, Charles C Thomas, 1958.

they reduce to a minimum their insensible pulmonary and cutaneous water loss.² Humans, in contrast, even when forming maximally concentrated urine, require some exogenous source of free water to replace continued insensible and minimal renal losses of water.

The day-to-day regulation of body water is dependent, therefore, on the kidneys and on a receptor system for signaling the relative need for water ingestion. This signal, thirst, plays an essential role in the regulation of the volume and tonicity of the body fluids. The sensation of thirst is dependent on excitation of the cortical centers of consciousness. Although the sensation of thirst may be modified somewhat by stimuli arising in the oropharynx or gastrointestinal tract, it is doubtful that in humans these local stimuli are of great importance.³

Hypothalamic Thirst Centers

Although thirst is a cortical or conscious sensation, it has been clearly established that there exist in the hypothalamus specific nuclear centers essential for the integration of various types of signals altering water ingestion.³⁻⁵ The local instillation of hypertonic solutions of chloride or direct electrical stimulation of this area in goats leads to profound polydipsia culminating in severe water intoxication, with an increase in body weight of as much as 40 percent. Destruction of these same centers causes pronounced hypodipsia or complete adipsia. These animals then develop profound hypertonic dehydration, but show no distinct inclination to drink. Stimulating and inhibitory impulses are transmitted from these centers to the cerebral cortex and consciousness, thereby transforming the need, or lack of need, for water into appropriate behavior. Furthermore, impulses of cortical or voluntary origin can readily condition the sensation of thirst and create what might be called the thirst *appetite*, or voluntary habits of drinking. These vary greatly from individual to individual, psychogenic polydipsia representing the extreme form of excessive ingestion. Conversely, in humans, injury to these centers, or the failure of hypothalamocortical impulses to stimulate thirst in a stuporous or unconscious patient, will cause inadequate water ingestion despite need.

The thirst centers lie in close proximity to the hypothalamic nuclei which regulate the production and release of antidiuretic hormone or arginine vasopressin. In view of this proximity, it

is surprising that pathologic processes do not produce simultaneous disturbances in thirst regulation and AVP release more frequently. Physiologically, these centers must be carefully integrated in the maintenance of normal total body water. It is not surprising, therefore, that the major stimuli for thirst also cause the release of AVP and the renal conservation of water—the *water repletion reaction*. Those stimuli which in-

TABLE 3.—Known Stimuli and Inhibitors of Thirst and the Release of Arginine Vasopressin

Stimuli

Osmotic

Contracted intracellular volume of osmoreceptor neurons due to hyperosmolality of extracellular fluids (water loss or infusions of hypertonic solutions of molecules that do not freely permeate the blood-brain barrier, such as sodium chloride or mannitol)

Nonosmotic

- Decreased arterial pressure (? pulse pressure) in carotid and, possibly, aortic baroreceptors, resulting from vascular or extracellular volume contraction or fall in cardiac output
- Decrease in tension in the left atrial wall and great pulmonary veins because of reduced intrathoracic blood volume (such as blood loss, quiet standing, upright position and positive-pressure breathing)
- Pain
- Psychosis
- Increased temperature of blood perfusing the hypothalamus
- Drugs (acetylcholine and other cholinergic drugs, morphine, barbiturates, nicotine, adrenergic agents), ? chlorpropamide, clofibrate, carbamazepine, cyclophosphamide, vincristine
- Carotid body chemoreceptor stimulation by hypoxia or hypercapnia
- Stimulation of the renin-angiotensin system

Inhibitors

Osmotic

Expansion of intracellular volume of the neurons of supraoptic hypophyseal nuclei secondary to hyposmolality of extracellular fluids (water ingestion)

Nonosmotic

- Increased arterial pressure (? pulse pressure) in carotid and, possibly, aortic baroreceptors secondary to vascular or extracellular volume expansion, with rise in cardiac output
- Increase in tension in left atrial wall and great pulmonary veins secondary to increased intrathoracic blood volume (such as hypervolemia, reclining position, negative-pressure breathing, immersion in water up to neck, acute cold exposure with peripheral vasoconstriction and shift of blood centrally)
- Occasionally, emotional stress
- Decreased temperature of blood perfusing the hypothalamus
- Drugs (alcohol, diphenylhydantoin, ? norepinephrine, narcotic antagonists [oxilorphan, butorphanol], ? anticholinergic drugs)
- ? inhibition of the renin-angiotensin system

hibit thirst also inhibit the release of AVP and therefore initiate water diuresis—the *water depletion reaction*.⁶

Thirst Stimuli and Inhibitors

The major physiologic stimulus for thirst is a decrease in total body water, with or without cellular dehydration (Table 3). The usual sensation of thirst, and its subcortical stimulus, in a normal person is the result of a 1 percent to 2 percent contraction of total body water with a comparable rise in the osmolality of body fluids and slight cellular dehydration. In more severe cases of water depletion, or water loss in excess of sodium, substantial cellular dehydration and hyperosmolality of all body fluids will occur. Cellular dehydration with pronounced thirst will also occur, even with an increased total body water, if hypertonic solutions of sodium chloride, glucose, mannitol and so forth, are given to the point of producing hyperosmolality of the body fluids. Of equal importance, the decrease in total body water may result from a primary iso-osmotic contraction of the extracellular phase, such as hemorrhage, vomiting, diarrhea, massive saline diuresis, or a rapid accumulation of ascites or intraluminal gastrointestinal fluid (*third space*).

Under these circumstances, thirst may be induced even in the face of hyponatremia and cellular overhydration and, in fact, may even aggravate the latter. Stricker has shown that cellular overhydration in association with, or at the expense of, extracellular volume will frequently produce severe thirst in rats.^{6,7} This hypovolemic thirst is permanently satisfied only when the extracellular or intravascular volume is repleted. Clinically, thirst may occur despite massive expansion of total body water or of interstitial fluid volume and despite hyponatremia, when *effective* (non-splanchnic) circulating blood volume, blood pressure and cardiac output are reduced, such as in severe congestive heart failure, cirrhosis of the liver (during the rapid accumulation of ascites or edema) and the nephrotic syndrome. Whenever a patient complains of thirst despite adequate ingestion of water, or when a deficit of water per se does not seem to exist, one should immediately consider the possibility of a diminished vascular or extracellular volume, or reduced cardiac output and blood pressure, or both.

Such *inappropriate* stimulation of thirst in patients with normal osmolality or hyposmolality may also occur with normal or elevated blood

pressure. In these situations there is overactivity of the renin-angiotensin system which in turn stimulates thirst. For example, intense thirst has been noted in patients with malignant hypertension or in hypertensive patients overtreated with diuretic therapy.

The exact mechanism(s) by which a reduction in vascular and extracellular volume stimulates the hypothalamic thirst centers is unknown. Although it is probable that pure water depletion with its accompanying hyperosmolality of body fluids and contraction of intracellular volume would affect the receptor cells of the hypothalamus directly, it is not obvious that iso-osmolar or hyposmolar contraction of the extracellular volume would do the same. However, because it is clear the latter can cause the release of AVP by stimulation of intrathoracic and carotid sinus baroreceptors (Table 3),⁸⁻¹³ these same receptors may well represent the receptors initiating the reflex arc responsible for nonosmolar stimulation of thirst. Recently, excellent studies have strongly implicated the renin-angiotensin system in the control of hypovolemic (hypotensive) thirst¹⁴⁻²¹ and in the release of antidiuretic hormone.^{18,22-29} The drinking produced by iso-osmotic depletion of intravascular volume,¹⁴ by hypotension accompanying beta-adrenergic activation with isoproterenol and by constriction of the aorta above the renal arteries¹⁹ is abolished by nephrectomy but survives sham nephrectomy and ureteral ligation.^{14,19} In hypotensive rats, in which the kidneys have been removed, renin administered systemically and angiotensin II given intracranially (anterior hypothalamus) caused the animals to begin drinking again.^{14,15,17,20} It, therefore, seems likely that low cardiac output, hypovolemia or hypotension, by stimulating the release of renin from the kidneys and the formation of angiotensin II, may cause thirst by a direct effect of the angiotensin II on the thirst regulatory centers in the hypothalamus. It is clear that any physiologic or pathologic change that causes a renal release of renin and the generation of angiotensin will stimulate or contribute to the stimulation of thirst and the release of antidiuretic hormone as well.

The exact neurotransmitters responsible for the activation of the hypothalamic component of thirst regulation are not known. However, several experiments in animals have shown that local hypothalamic application of cholinergic and adrenergic agents as well as heat and cold can profoundly influence both their relative drives for

food and water and their fluid excretion.³⁰⁻³³ Recently, local administration of prostaglandin E (PGE) has also been found to stimulate both thirst and AVP release.³⁴ Furthermore, PGE augments the action of either angiotensin or hypertonic saline on central release of AVP.^{35,36} These data suggest a possible central role for PGE in the modulation of thirst and AVP release.

Warming of the hypothalamus leads to a decrease in food intake, an increase in water intake and a decrease in urinary output, the water repletion reaction. Cooling of the hypothalamus leads to the reverse reactions. Similarly, rats, during dehydration, automatically decrease their ad libitum food intake to maintain proper integration of total body water and solute. Cholinergic agents (carbachol) applied directly to the hypothalamus stimulate the water repletion reaction and inhibit food intake. These reactions can be blocked by anticholinergic drugs, whereas the application of the alpha-adrenergic, norepinephrine, stimulates food intake, inhibits water intake and enhances urinary volume, the water depletion reaction. In addition, systemic administration of norepinephrine directly inhibits the action of AVP on the nephron. In contrast to norepinephrine, beta-adrenergic agents such as isoproterenol hydrochloride (Isuprel), when applied locally, stimulate the classic water repletion reaction, which can be completely inhibited by the simultaneous application of the beta-adrenergic blocking drugs. Finally, Fitzsimons and Setler³⁷ have clearly shown that interference with catecholaminergic systems in the diencephalon (preoptic region) by local instillation of catecholamine antagonists substantially inhibited angiotensin-induced drinking, but had little effect on cholinergic-induced drinking. The latter was completely inhibited by atropine. These findings suggest that the final common pathway for the central effect of angiotensin on the thirst mechanism involves stimulation of the adrenergic (beta) neurotransmitters of this area of the hypothalamus.

These experimental observations should be considered when the eating, drinking or urinary flow patterns of a given patient seem to be altered while the patient is receiving cholinergic or adrenergic drugs or their inhibitors, or in clinical situations in which the renin-angiotensin II system is stimulated.

Various organic hypothalamic lesions may alter thirst to the extent that adipsia, severe hypertonic dehydration and sustained hypernatremia may

occur.^{37,38,40} These lesions may be traumatic or inflammatory (such as encephalitis), or may involve eosinophilic granuloma, histiocytosis, internal hydrocephalus, craniopharyngioma, pinealoma and, in rare cases, so-called essential hypernatremia with a defect in thirst regulation in which no specific lesion may be found. In some patients diabetes insipidus or anterior pituitary insufficiency, or both, may also be present.

Polydipsia and polyuria are common symptoms in patients with chronic hypercalcemia or hypokalemic syndromes. Although a pronounced defect in the ability of the kidneys to concentrate urine may contribute to the polyuria, we have noted that polydipsia may be present despite the patient's ability to form a moderately hypertonic urine (400 to 700 mOsm per liter), and that the polydipsia may disappear after correction of hypercalcemia and hypokalemia long before any basic improvement in the concentrating abnormality has occurred. These observations suggest that the electrolyte disorder may cause a primary disturbance in thirst regulation. Recently, Berl and co-workers³⁹ have confirmed this clinical observation by noting that in potassium-depleted rats a primary polydipsia was responsible for the polyuria rather than the concentrating defect. Because potassium depletion now has been shown to be a strong stimulus to enhanced PGE synthesis, it is possible that this polydipsia is PGE induced. As calcium ion is a specific biologic antagonist of potassium, it would not be surprising to find that hypercalcemia also stimulates PGE synthesis.

Arginine Vasopressin, Antidiuretic Hormone

Arginine vasopressin (AVP) acts on the renal tubule and, in doing so, has a fundamental role in the homeostatic regulation of the volume and osmolality of the body fluids in mammals. The integration of AVP release with the thirst mechanism is necessary to assure the maintenance of a normal water content and osmolality in the body fluids. It is readily apparent that a distortion in the thirst mechanism without a corrective response in the renal handling of water, or a primary disorder in the renal handling of water without an appropriate corrective response in the thirst mechanism, will result in a serious distortion of the volume and osmolality of body fluids.

The Neurohypophyseal System

In mammals the neurohypophyseal system responsible for the synthesis, storage and release of

AVP consists of a group of specialized hypothalamic nuclei (supraoptic and paraventricular) and the neurohypophyseal tract, made up of the axons originating from these nuclei and terminating in the pars nervosa or posterior lobe of the pituitary.

A discrete neural lobe with its systemic blood

supply first appears phylogenetically in Amphibia (Figure 1). These semiterrestrial vertebrates required a carefully regulated system for the retention and elimination of water; the neural lobe supplied the hormone (arginine vasotocin) for this purpose. The neural lobe in the earlier vertebrates appeared to be a structure totally surrounded by the anterior pituitary (Figure 1), and the peptides formed in this area probably served primarily as releasing factors for anterior pituitary hormones.

Neurohypophyseal Peptides

However, despite the absence of a distinct neural lobe, biologically active octapeptides have been identified in the neurohypophyseal extracts of lower vertebrates; these are listed in Table 4.⁴¹ Only three of these principles were considered by Sawyer and others to be antidiuretic hormones: arginine vasopressin, lysine vasopressin and arginine vasotocin. All three contain a basic amino acid in the penultimate position of the side chain, a molecular configuration responsible for their strong antidiuretic and vasopressor properties. The remaining natural peptides resemble oxytocin more closely in that they contain a neutral amino acid in the 8-position of the side chain. These have relatively weak vasopressor and antidiuretic effects. The exact chemical structure of arginine vasopressin and lysine vasopressin was determined by du Vigneaud and his associates after they defined the structure of oxytocin. As in the case of oxytocin, they confirmed the exactness of the chemical configuration by synthesizing peptides with biologic properties identical to those of the natural principles that they had isolated from beef and hog posterior pituitaries.

Arginine vasopressin appears to be present in representative species from most of the major groups of mammals, including the egg-laying monotremes and the marsupials. Lysine vasopressin has been found only in pituitaries from members of the division Suina (suborder Suiformes), which contains pigs, peccaries and hippopotami. All members of the other suborder of Artiodactyla that have been examined, the ruminants, appear to secrete arginine vasopressin. Although lysine vasopressin occurs in all three living families of the Suina, it has not entirely replaced arginine vasopressin. One of five pituitaries from the wild boar contained arginine vasopressin as well as lysine vasopressin. This species is believed to be ancestral to the domestic pig, suggesting that the

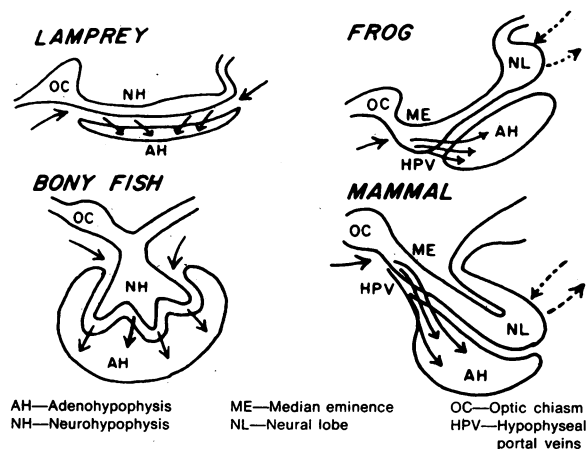


Figure 1.—Highly schematic diagrams of the hypophyseal circulation in four vertebrate types. Solid arrows represent blood flow from neurohypophysis to adenohypophysis. Broken arrows represent the blood flow to and from the neural lobe. (From Sawyer WH, et al: *Circulation*, 1960, Suppl, part 2, 21:1028. Reproduced with permission of the author and The American Heart Association.)

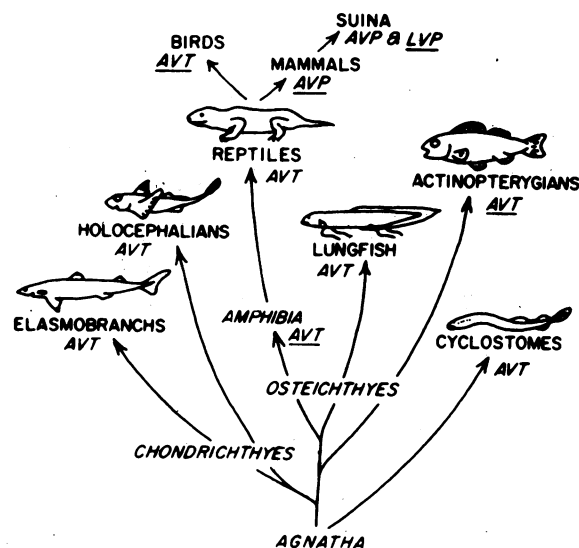


Figure 2.—The distribution of antidiuretic-vasopressor neurohypophyseal principles among the various major groups of vertebrates. AVP=arginine vasopressin; LVP=lysine vasopressin; AVT=arginine vasotocin. Underlining of the abbreviation indicates that the peptide has been chemically identified in at least one member of the phyletic group. (Reproduced with permission from Sawyer.⁴²)

TABLE 4.—*The Known Active Neurohypophyseal Hormones of Vertebrates**

Peptide	<u>Positions of Amino Acids†</u>			Where Found
	3	4	8	
Antidiuretic vasopressor (basic) principles:				
Arginine vasopressin	Phe	Gln	Arg	Most mammals
Lysine vasopressin	Phe	Gln	Lys	Suina
Arginine vasotocin	Ile	Gln	Arg	All (?) vertebrates
Oxytocin-like ("neutral") principles:				
Oxytocin	Ile	Gln	Leu	Mammals, ratfish
Mesotocin	Ile	Gln	Ile	Nonmammalian tetrapods, lungfishes
Isotocin	Ile	Ser	Ile	Ray-finned fishes
Glumitocin	Ile	Ser	Gln	Rays
Valitocin	Ile	Gln	Val	Spiny dogfish (? other sharks)
Aspartocin	Ile	Asn	Leu	Spiny dogfish (? other sharks)

*From Sawyer WH: *Am Zool* 17:728, 1977.†Common structure: Cys-Tyr-3-4-Asn-Cys-Pro-8-Gly(NH₂)

ability to synthesize arginine vasopressin may have been lost by domestic pigs as an indirect result of selective breeding. Because the synthesis of vasopressin is almost certainly genetically determined, this implies that the gene allowing lysine-vasopressin synthesis originated by mutation, occurring in a species ancestral to the Suina. Figure 2⁴¹ shows the distribution of antidiuretic-vasopressor neurohypophyseal hormones among the various groups of vertebrates. In general, the bony fishes as well as the teleosts have only one neurohypophyseal hormone, vasotocin. Some teleosts and holocephalians contain a second active principle called isotocin (Table 4). This peptide may also be present in lungfish. The elasmobranchs may have two different neutral peptides: arginine vasotocin and glumitocin (Table 4).

Vasotocin, having the ring of oxytocin and side chain of vasopressin, produces both antidiuretic and milk-ejection effects when injected into mammals, but it is not nearly as potent in this regard as vasopressin. Vasotocin enhances water transport in the nephrons of birds, reptiles and amphibians but not in bony fishes, cartilaginous fishes or cyclostomes; it appears to enhance sodium excretion in these latter vertebrates (Table 5).⁴¹ Extraordinarily low doses of vasotocin (10⁻¹⁶M) have been shown to have potent effects on the contraction of the oviduct of the mud puppy (*Necturus maculosus*) during the spring.⁴² This action of vasotocin reflects its oxytocic as opposed to its vasopressor properties.

Vasotocin has also been identified in the posterior pituitary of fetal mammals as well as in the pineal glands and subcommissural organs of adult mammals.⁴³⁻⁴⁵ Its function in mammals is not

TABLE 5.—*Responses to Synthetic Arginine Vasotocin Described as Occurring in Representatives of the Major Phyletic Groups of Vertebrates**

Group	Urine Flow	Filtration Rate	Sodium Excretion	Permeability to Water	
				Skin	Bladder
Mammals	—	0	0, +		
Birds	—	0			
Reptiles	—	—	—	..	0
Amphibians	—, 0	0, —	0, —	+, 0	+, 0
Lungfishes	+	+	+	0	
Teleosts	+	+	+	0	
Cyclostomes	0	..	+	0	

+ = increase; — = decrease; 0 = no change. Where two signs appear species differ in their responses to arginine vasotocin.

*From Sawyer WH.⁴²

known although it has been shown to have potent antigonadotrophic and prolactin-releasing effects after intravenous or intracerebroventricular injection.^{46,47}

Osmoreceptors for AVP Release

For sometime it was believed that the magnocellular neurons in the hypothalamus were capable of sensing changes in plasma osmolality, thereby releasing vasopressin. There is now evidence that there are multiple osmosensitive areas outside the supraoptic and paraventricular nuclei. Using the technique of microinjection of hypertonic solutions into various locations in the central nervous system, Peck and Blass⁴⁸ identified separate osmoreceptors mediating thirst and vasopressin release. The osmoreceptor centers were scattered within the preoptic areas and anterior hypothalamus. The electrophysiologic studies of Hayward and colleagues have suggested that there are different functional types of osmosensitive cells (specific and nonspecific) and that these

cells are one synapse removed from the neurosecretory neurons. Van Gemert and co-workers⁴⁹ have shown that lesions made in the medial preoptic area, with the supraoptic nuclei spared, resulted in polyuria and impaired vasopressin release. These observations have pointed to regulatory centers for thirst and AVP release outside the neurosecretory nuclei.

Formation and Storage of AVP

The posterior lobe of the pituitary is the storage site for the AVP and oxytocin formed in the nerve cell bodies in the supraoptic and paraventricular nuclei. Although the content of oxytocin and AVP in the posterior pituitary are approximately equal, and frequently both hormones are released together, selective release appears to occur when certain physiologic stimuli are applied; for example, hemorrhage causes AVP release, whereas distention of the cervix and suckling cause the release of oxytocin. In autopsy material we have found the AVP content of the posterior pituitary in humans to be 85 ± 15 units per mg relative to the *United States Pharmacopeia* International Standard, and others have reported approximately 15 USP units per whole human gland.

Electron microscopic and other histologic studies of the neurohypophyseal system strongly suggest that the membrane-bound neurosecretory granules that are formed by the nerve cell bodies of the supraoptic nuclei are carried by axoplasmic streaming down the axons which terminate as bulbous expansions on the basement membranes of the capillaries in the posterior lobe. The granules containing the peptide hormones and their carrier proteins gradually enlarge as they descend along their intra-axonal path to their storage site in the posterior lobe.

Rosenfeld,⁵⁰ in 1940, and van Dyke and colleagues,⁵¹ in 1942, isolated a protein from the posterior lobe of the ox pituitary gland. Bioassay showed constant oxytocic pressor and antidiuretic activities. This material, often called the van Dyke protein, was thought to contain the biologically active principles of the posterior lobe. In the mid-1950's, Acher and associates^{52,53} noted the relationship between the peptide hormones oxytocin and vasopressin and the cystine-rich van Dyke protein (extracted from bovine pituitaries). They found that oxytocin and vasopressin could be reversibly separated from the larger binding proteins, and they suggested the name neurophysin for the hormone-free proteins. The findings of

Acher and his associates were confirmed by Ginsburg and Ireland.⁵⁴ The latter investigators, as well as Dean and Hope,⁵⁵ found that the neurophysins were stored in the same subcellular organelles (neurosecretory granules) as oxytocin and vasopressin, and they have come to be regarded as the physiologic *carrier proteins* for the intraneuronal transport of the hormones from their site of synthesis in the hypothalamus to the neurohypophysis. Neurophysins, isolated from the posterior pituitary glands of rats,⁵⁶ porcines,⁵⁷ bovines^{57,58} and humans⁵⁹ are relatively small protein molecules, containing 18 different amino acids per mole and varying in molecular weight from 9,500 to 10,500. The evidence is conclusive that there is a specific oxytocin-binding as well as a specific vasopressin-binding neurophysin,^{56,57,59-62} each mole of protein binding one mole of vasopressin or oxytocin. It is of considerable interest that neurohypophyseal extracts from homozygous rats with hereditary hypothalamic diabetes insipidus (Brattleboro strain) contain normal amounts of oxytocin and oxytocin-binding neurophysin, but no vasopressin or its neurophysin.⁵⁶ The finding of hormone-specific neurophysins may contribute to a better understanding of the selective release of oxytocin and vasopressin.

Sachs and co-workers,⁶³ using the technique of injection of cysteine labeled with sodium sulfate S 35 into the hypothalamus, with subsequent *in vitro* isolation of vasopressin and neurophysin labeled with sodium sulfate S 35, have clearly documented that the biosynthesis of labeled vasopressin is paralleled by the formation of labeled neurophysin. Sachs and another group of associates⁶⁴ confirmed these observations, using supraoptic neurosecretory neurons of the guinea pig in organ culture, thereby suggesting that protein and hormonal peptide may arise from a common precursor. On the basis of these observations and their own studies, Burford and colleagues⁶⁶ have suggested that the relationship of each neurohypophyseal hormone and its relevant neurophysin is analogous to that of insulin and C-peptide. By this analogy, whereas in the beta-cell of the pancreatic islet, proinsulin is split to give insulin and C-peptide in the hypothalamo-neurohypophyseal cell, provasopressin and pro-oxytocin would give rise to vasopressin and vasopressin-neurophysin, and oxytocin and oxytocin-neurophysin, respectively. In 1967 Friesen and Astwood⁶⁵ suggested that these binding proteins might

be secreted into the circulation because after dehydration or administration of hypertonic saline in rats, they observed a parallel decrease in the vasopressin and neurophysin content of the extracted posterior pituitary glands. Shortly thereafter, Fawcett, Powell and Sachs⁶⁶ were able to extract neurophysin-like proteins from cavernous sinus blood following hemorrhage. Because of the development in several laboratories^{61,62,67-69} of a sensitive and specific radioimmunoassay for the neurophysins, the evidence is now indisputable that neurophysin is not confined to the neurohypophysis but is released into the circulation in response to various physiologic stimuli that cause the release of vasopressin and oxytocin.

In recent years, Sachs and associates have attempted to delineate the steps in the biosynthesis of vasopressin in the hypothalamo-neurohypophyseal complex of dogs and guinea pigs by use of isotope-labeling techniques.^{70,71} They have proposed a precursor model for vasopressin biosynthesis in which the biosynthesis would occur solely in the perikaryon of the neurons or nerve cell body of the supraoptic nucleus. Hormone synthesis is presumably initiated on the ribosomes via pathways common to the biosynthesis of other proteins and peptides, involving transfer RNA, messenger RNA, and so forth. However, Sachs and co-workers have proposed that the biosynthesis of vasopressin first leads to a bound, biologically inactive form (provasopressin) as part of a precursor molecule, and that the appearance of the biologically active nonapeptide occurs at a time and place removed from the initial biosynthetic events.

Conceivably, the release of the nonapeptide from the precursor molecule would take place during the formation and maturation of the neurosecretory granule. The most convincing evidence in favor of a *precursor molecule model* has come from labeling experiments carried out *in vitro* with guinea pig hypothalamo-median eminence tissue. As in the case of the *in vivo* isotope experiments, the incorporation of amino acids labeled with sodium sulfate S 35 and tritiated water into vasopressin was preceded by a lag period of about an hour. If the slices were removed from the initial incubation medium after the first hour of incubation and reincubated in fresh buffer, labeled hormone appeared under conditions which precluded further *de novo* peptide bond synthesis. Furthermore, the release of labeled vasopressin from a labeled precursor can take place in a cell-

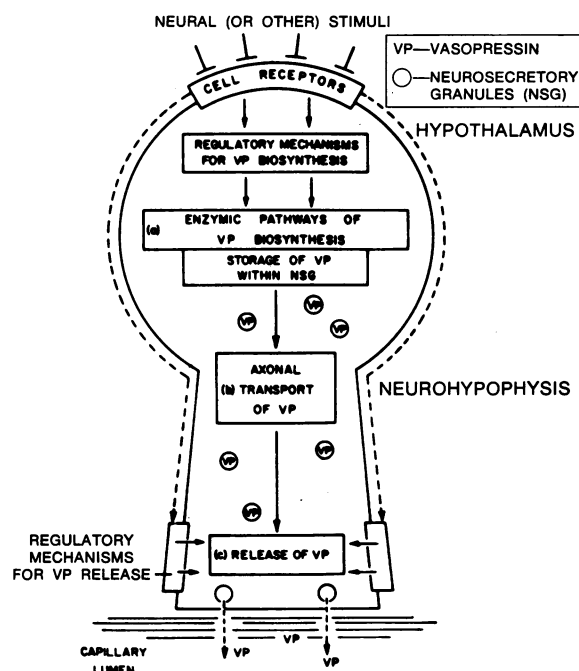


Figure 3.—Schematic representation of several intermediate stages involved in the neurosecretory function of the vasopressin-producing cells of the mammalian hypothalamo-neurohypophyseal complex. (Reproduced with permission from Sachs H, et al, *In* Stutinsky F (Ed): *Neurosecretion*. Berlin, Springer-Verlag, 1967.)

free system. Although the biosynthesis of labeled precursor formed during the first hour of slice incubation was inhibited by puromycin, the release of hormone from precursor was not inhibited by this drug.

Figure 3 is a schematic representation of Sachs' concept of the intermediate stages involved in the neurosecretory function of the vasopressin-producing cells of the mammalian neurohypophysis. Using the radioisotope-labeling model of hormone biosynthesis, Sachs and his associates consistently found that when intact guinea pigs were deprived of water for four days before removal of neurohypophyseal tissues, the neurohypophyseal tissues incorporated two to five times more radioactivity in the newly synthesized vasopressin than in the same tissues from guinea pigs with free access to water. These findings suggest that the prolonged maintenance of nerve impulses effective in the release of vasopressin is probably also translated into sustained synthesis of the hormone. Therefore, it is likely that all stimuli which enhance release of AVP also enhance its synthesis (Table 3). It is of great interest that a species of rat (Brattleboro strain) with congenital vasopressin-

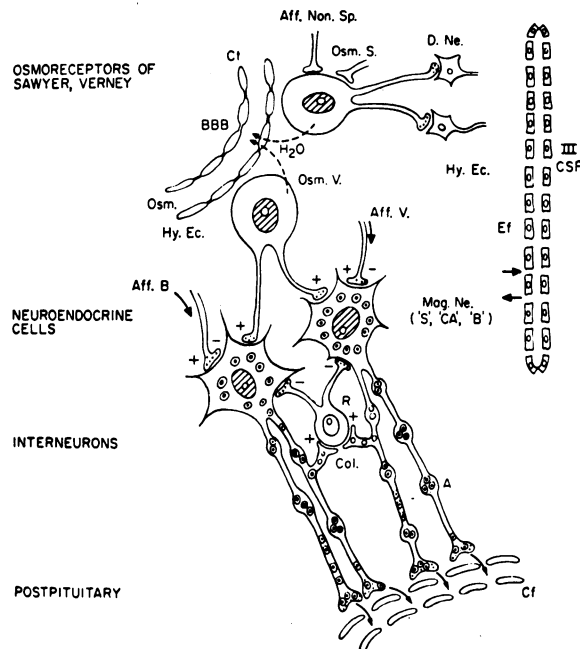


Figure 4.—Schematic drawing of the hypothalamo-neurohypophyseal system. The osmoreceptors of Verney (Osm. V.) are seen as anatomically discrete cells whose axons project to the magnocellular neurons (Mag. Ne.) in the hypothalamus. The latter cells then project to the posterior pituitary. Also shown are the osmoreceptors of Sawyer (Osm. S.) which respond to changes in osmolality as well as to noxious stimuli (Aff. V.). Axons from afferents related to behavioral stimuli (Aff. B.) are shown projecting to the magnocellular neurons. (Reproduced with permission from Hayward JN: Neurohumoral regulation of neuroendocrine cells in the hypothalamus, *In* Lederis K (Ed): *Recent Studies of Hypothalamic Function*. Basel, Karger, 1974, pp 166-179.)

sensitive diabetes insipidus has enormously enlarged supraoptic nuclei which, on chemical analysis, contain little or no AVP.⁷² Therefore, although the hormone-forming neurons exist, some specific chemical defect prevents their synthesis of AVP.

The posterior lobe receives its blood supply from and secretes its hormone directly into the systemic circulation.

Neurophysiology of AVP Secretion

The introduction of methods capable of recording electrical activity in a single magnocellular neuron and of confirming the identity of these cells by antidromic stimulation has provided many insights into the regulation of neurosecretion.⁷³ Hayward and co-workers identified two distinct cell types on the basis of their response to infusion of hypertonic saline.⁷⁴ Cells with a monophasic pattern of activity that were stimulated by hyperosmolality, but were not af-

fected by noxious stimuli, were thought to be the specific osmoreceptors of Verney that are involved in AVP release; cells with a similar specificity, but having a biphasic response pattern, were felt to be the magnocellular neurons. Alternately, cells that responded to either osmotic or noxious stimuli were designated osmoreceptors of Sawyer (Figure 4). It was speculated that these were important in the drinking and behavioral aspects of osmoregulation. In other studies, the magnocellular neurons were characterized on the basis of their firing pattern into the three following groups: silent, phasic (bursting) or continuously active. Several investigators have described modification in the firing patterns of these neurons in response to various stimuli.⁷⁵⁻⁷⁷ This suggests that the firing pattern is, in part, a reflection of the state of activity of the neuron. There is evidence, however, that a phasic firing pattern appears to be associated with vasopressin-secreting cells and a random firing pattern with oxytocin-secreting cells.⁷⁷ Findings from such neurophysiologic studies have suggested that the specific neurons that synthesize and secrete vasopressin and oxytocin are distributed in both the supraoptic and paraventricular nuclei (Figure 5). At present, no specific functions can be attributed totally to one or the other nucleus.

Considerable progress in unraveling the physiology of AVP secretion has been made by using techniques for histochemical identification of neurosecretory neurons. The use of specific antibodies to arginine vasopressin, oxytocin and neurophysins has facilitated histologic identification not only of magnocellular (secretory) neurons, but has also resulted in delineation of additional pathways of AVP secretion in the hypothalamus. Immunohistochemical studies of hypothalamic nuclei have confirmed the intermingling of vasopressin- and oxytocin-secreting neurons in both the supraoptic and paraventricular nuclei as described above.⁷⁸ The bulk of the evidence from such studies seems to support the concept of one neurosecretory neuron producing either vasopressin and its neurophysin or oxytocin and its neurophysin.^{78,79} These studies have also permitted identification of fiber tracts from the neurosecretory nuclei other than those traversing the pituitary stalk. One of these tracts projects to the zona externa of the median eminence and appears to be responsible for the very high concentration of AVP found in hypophyseal portal blood.⁸⁰ Staining in this area was greatly increased in rats

in which the adrenal glands had been excised and in which there were substantial elevations in the level of plasma AVP.⁸¹ These observations suggest a possible role for AVP along with other hypothalamic factors in modulating corticotropin secretion. There is also evidence suggesting transport of AVP from the neurosecretory neurons to the third ventricle. Studies in our laboratory have shown that hemorrhage provokes a pronounced rise in both plasma and cerebrospinal fluid (CSF) concentrations of AVP, though to a lesser extent in the latter.⁸² Such elevations did not occur after intravenous administration of AVP, which suggests that AVP was actively secreted into the CSF. This is supported by the failure of intravenously administered tritiated oxytocin to cross the blood-CSF barrier.⁸³ The vasopressin present in the CSF may play some role in memory consolidation. De Wied and co-workers have shown that injection of vasopressin into the third ventricle increased resistance to the extinction of a conditioned avoidance response. Memory consolidation was inhibited by injection of antivasopressin antiserum into the third ventricle.⁸⁴ They suggested that hypothalamic pathways can transport AVP to the infundibular recess of the third ventricle where it can be released into the CSF and influence memory processes related to behavioral homeostasis.

Secretion or Release of AVP

The release of AVP into the circulation, regardless of the stimulus, is dependent on depolarizing impulses arising in the supraoptic nuclei and transmitted down the axon to the hormone-storing terminal ending in the posterior lobe.⁹ The *pores* in the capillaries of the neural lobe have a diameter of approximately 30 to 75 m μ or 300 to 750 Å, whereas the intact neurosecretory granules in the neural lobe have a diameter of 1,600 to 5,000 Å.⁹ This difference in size suggests that the intact granules cannot be released as such into the circulation. However, the recent elegant electron microscopic and biochemical studies of Douglas and associates⁸⁵⁻⁸⁸ have clearly reconciled this difference as well as defining the nature of the cellular events involved in the secretion of posterior pituitary hormones.

Earlier studies^{85,89,90} suggested that on stimulation of the neurohypophysis the granules stored in the nerve ending release their contents in the form of a molecular dispersion. This dispersal allows the hormones to traverse the cytoplasm and plasma membrane of the nerve ending to

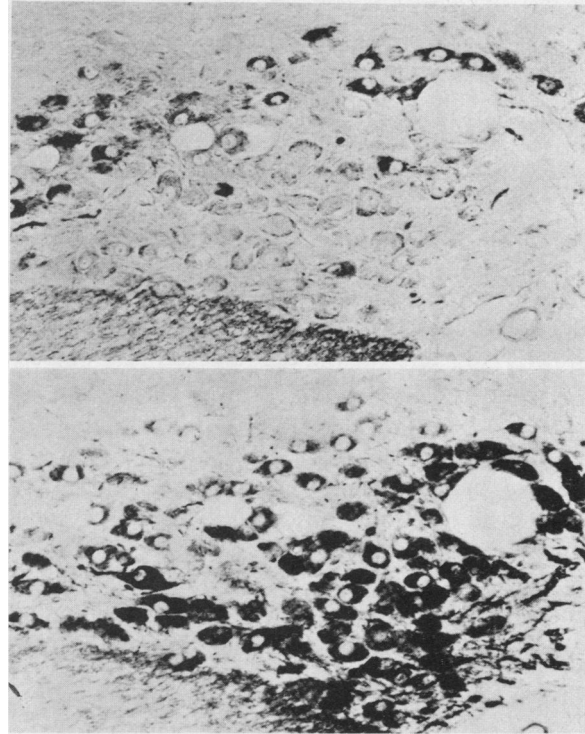


Figure 5.—Adjacent 5 μ M coronal sections of the supraoptic nucleus of a normal rat reacted for oxytocin (**top**) and vasopressin (**bottom**) by the immunoperoxidase technique. Oxytocin-containing cells are concentrated in one dorsal part of the nucleus whereas vasopressin cells are found scattered throughout the nucleus with most intense staining seen in the ventral portion. Some cells are reactive for both hormones (magnification $\times 260$). (Reproduced with permission from Zimmerman EA, Defendini R: Hypothalamic Pathways Containing Oxytocin Vasopressin and Associated Neurophysins. International Conference on the Neurohypophysis, Nov 14-19, 1976, Key Biscayne, Florida. Basel, S Karger AG, 1977.)

reach the extracellular space and from there the capillary lumen. Douglas and associates⁸⁵⁻⁸⁸ point out that all previously proposed schemes have shared a common notion of an intracellular mechanism that is responsible for the dissociation of the hormones from their intracellular sites of storage or binding and their subsequent transport, possibly by molecular diffusion across the various plasma membrane barriers, into the circulation. These investigators⁸⁵⁻⁸⁸ have convincingly concluded from their electron microscopic evidence that the neurosecretory granules discharge their contents directly onto the cell exterior by the process of exocytosis, beginning with fusion of the granules with the plasma membrane, and subsequent formation of an opening that communicates with the extracellular space. This mode of secre-

tion is most consistent with observations^{61,62,67-69} that the hormone-binding protein neurophysin in the neurosecretory granules escapes into the circulation with the hormones when the posterior pituitary gland is stimulated.

From their excellent studies Douglas and colleagues⁸⁵⁻⁸⁸ have further proposed that the *synaptic vesicles*, which accumulate at the nerve endings after the release of AVP and oxytocin, arise as by-products of secretion by exocytosis. These electron-lucent structures, about a fourth to a fifth the diameter of the neurosecretory granules, were called synaptic vesicles by Paley⁹¹ because their size, appearance and distribution were similar to those of the synaptic vesicles in ordinary neurons. Paley and others suggested that they participate in hormone release. Douglas has conclusively shown that these synaptic vesicles form by inward budding endocytosis of the cell surface, mainly at the base of the exocytotic pit. The microvesicles so formed are thought to move from the membrane, aggregate and migrate centripetally in the fiber away from the terminal region of the membrane engaged in hormone extrusion. Through this mechanism, the *surplus* plasma membrane, made available by the incorporation of the membrane of the neurosecretory granule into that of the axon terminal can be disposed of.

Douglas and Poisner have shown that when the neural lobe is incubated *in vitro* and stimulated (depolarized) either electrically or by high extracellular concentrations of potassium, the actual release of the hormone is strongly calcium ion dependent.⁹² Depolarization caused an increased uptake of calcium chloride Ca 45 by the neural lobe, and the secretion rate waned with the calcium content of the extracellular environment. They proposed the following steps: (1) propagation of impulse, after specific stimulus, down the neurohypophyseal stalk from supraoptic nuclei, (2) depolarization of neurosecretory axon terminals by impulses, (3) influence of calcium ion across the axon's plasma membrane and (4) activation of some calcium-dependent exocytotic process leading to hormone extrusion.

An extremely important concept regarding the release of large amounts of AVP from the neurohypophysis has been derived from the experiments of Sachs and associates.⁹³ Acute hemorrhagic hypotension is one of the most powerful stimuli for the immediate release of antidiuretic hormone. Within a few minutes after reducing the blood pressure of dogs to 50 mm of mercury by bleed-

ing, there is a rapid discharge of arginine vasopressin into the blood. In the presence of a maintained stimulus of hemorrhagic hypotension, the initial secretory response is not sustained beyond the first few minutes of hemorrhage. Reinfusion of the shed blood, after 30 minutes of hemorrhage, restored the arterial pressure and the blood AVP concentration to relatively normal values. A second hemorrhage an hour later failed to raise the concentration of AVP in the blood beyond the level observed just before blood reinfusion. Direct analysis of the pituitary gland showed that the decline in the release of AVP during hemorrhage could not be attributed to exhaustion of the pituitary content of AVP. Furthermore, AVP release did not appear to be a simple function of the total hormone content of the gland. Nevertheless, this attenuation of AVP release appears to reside, in part, at the level of the pituitary. Pituitaries taken from bled dogs released much less AVP *in vitro* in response to electrical or potassium stimulation than pituitaries from animals that had not been bled. Electron microscopic studies showed that the fine structure of the neural lobes of intact and bled dogs was similar in appearance, and there was no difference in the isolated specimens. Analogous results were obtained under circumstances (carotid perfusion and section of the vagi) in which hemorrhage failed to elicit an appreciable discharge of AVP.

As a working hypothesis, it is suggested that the pool of neurohypophyseal AVP is heterogeneous and that about 10 percent to 20 percent of the total hormone content of the gland can be readily released.⁹³ Once this smaller pool of hormone, which can be quickly released, has been discharged, the neurohypophysis continues to release arginine vasopressin in response to appropriate stimuli, but at a greatly reduced rate. Analysis of the amount of hormone remaining in the pituitary at the end of these experiments gave values that ranged from 2,500 to 7,000 mU of vasopressin per gland. There was no apparent correlation between the amount of AVP released during the second hemorrhage and the amount of hormone found in the gland.

Quantitative Aspects of Secretion

From studies using bioassays of the hormone or its infusion at varying rates in water-load in humans,⁹⁴⁻⁹⁶ one may conclude that in the normal ambulatory adult human, mild, sustained osmotic and nonosmotic stimuli cause the release of ap-

proximately 150 μU per minute per m^2 of AVP into the circulation. This is associated with a urinary osmolality of 500 to 1,000 mOsm per liter. During sustained dehydration (24 to 48 hours), with maximally concentrated urine, there is probably a threefold to fivefold increase in AVP release.^{10-12,94,96,97} Therefore, in a 24-hour period, a normal dehydrated human (1.73 m^2) will secrete between 1 and 2 units while maintaining a maximally concentrated urine. Stimuli such as severe pain, hemorrhage or an acute decrease in cardiac output may cause a 50-fold to 100-fold increase in the rate of secretion.^{8,95} For orientation it may be noted that the commercially available aqueous Pitressin contains 20 pressor units per ml of vasopressin, whereas Pitressin Tannate in Oil contains 5 pressor units per ml; 1 mg of the pure nonapeptide contains approximately 400 to 500 units of vasopressin.

The direct assay of ADH in the plasma of normal subjects in various states of hydration and the simultaneous measurement of plasma and urinary osmolality allow one to determine the relationship between concentrating ability and a given level of AVP in the circulation.^{8,10,12,94,96} Segar and Moore¹² and Moore¹¹ plotted this relationship in a large number of subjects, showing the curve of values from undetectable levels during water diuresis to concentrations as high as 20 μU per ml during 24 to 48 hours of dehydration. They found that in the state of normal hydration, at AVP concentrations of 1.5 to 2.0 μU per ml, the urinary-to-plasma (U/P) osmolar ratios varied from 1:1 up to a high of 4:1. The sigmoid nature of their curve (Figure 6) suggests that small changes of

between 1 and 3 μU per ml in plasma AVP will cause relatively large changes in the U/P osmolar ratio, whereas urinary concentrations greater than 4 μU per ml will change only slightly as circulating AVP levels approach their highest value during dehydration.¹² A similar plot of plasma AVP versus urine osmolality by Thomas and Lee⁹⁸ shows that urine osmolality varies as a linear function of log plasma AVP. This is in keeping with the log-dose response curve for AVP versus renal medullary adenylate cyclase. Therefore, from this curve and other available data,^{10-12,94,96,99} it is apparent that maximal renal conservation of water can be achieved by the release of only moderate amounts of AVP. Higher rates of secretion, which would increase the plasma AVP concentration above 4 μU per ml, would not influence further the maximal concentrating capacity in the dehydrated subject. This ability to produce the most concentrated urine at rates of AVP secretion far below maximal levels is due to the increased efficiency of the vascular and countercurrent mechanisms in the kidney that are responsible for the production of the hypertonicity of the renal medulla. This intrarenal adaptation allows for a further renal conservation of water without the added need for further secretion of AVP. This is strikingly apparent in the experiments of Valtin⁷² and Miller and Moses.⁹⁹ They studied rats with congenital diabetes insipidus that were unable to form AVP, yet in which progressive dehydration caused the urine osmolality to rise from its usual hypotonic level of 150 to 200 mOsm per liter to a peak of 1,100 to 1,200 mOsm per liter. Their findings

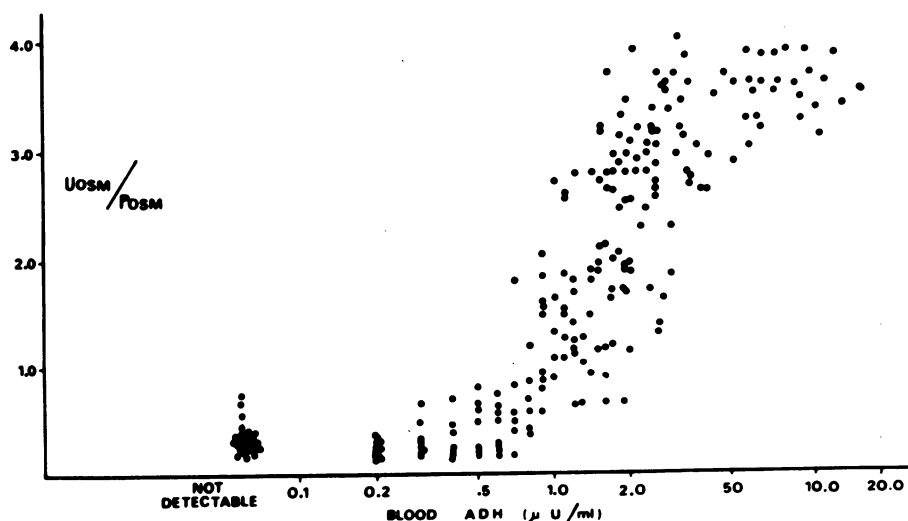


Figure 6.—Relationship between blood antidiuretic hormone concentration and urinary to plasma osmolar ratio (Uosm./Posm.) in humans; 203 pairs of data are shown. (Reprinted from Moore WW: *Federation Proceedings* 30:1387-1394, 1971.)

emphasize the importance of nonhormonal factors in the production of a maximally concentrated urine.

Osmotic and Nonosmotic Stimuli to AVP Secretion

Table 3 lists the known stimuli and inhibitors of the release of AVP. The final common pathway for all of these, regardless of their nature, is the nerve cell bodies of the hypothalamic supraoptic nuclei. The rate of hormone release at any moment will be controlled by the algebraic sum of the stimulating and inhibiting impulses impinging on the final common pathway. From this concept it is easy to see how the same osmotic stimulus—for example, a given increase in effective plasma osmolality—can be associated with different rates of secretion of AVP, depending on the other impulses of nonosmotic origin impinging on the supraoptic nuclei. The most common physiologic factor altering the osmolality of the blood is water depletion or water excess. The former causes a rise in the tonicity of all body fluids and cellular hydration, whereas the latter causes the reverse. The osmoreceptor neurons, in common with all body cells, share in this change in intracellular volume. The latter is probably responsible for altering the electrical activity of these neurons and, therefore, the release of AVP. If the tonicity or osmolality of all body fluids is increased by the administration of a solute such as urea that freely penetrates all body cells, no change in intracellular volume will occur and AVP release will not be stimulated. In contrast, an increase in the osmotic pressure of only 2 percent, caused by the administration of hypertonic saline during a water diuresis, will initiate the immediate release of a quantity of AVP sufficient to cause a pronounced antidiuresis (Figure 7).^{100,101} On the other hand, a comparable decrease in osmotic pressure, secondary to a water load, will stop the release of AVP and produce a maximal water diuresis unless this release is maintained by some nonosmotic stimulus (Figure 8). The concept of osmotic control of AVP and its subsequent effect on the nephron, painstakingly developed in experiments in dogs by Verney and associates, was presented in Verney's outstanding Croonian lectures.¹⁰² These represent the most fundamental work in this field and provide a lucid, exciting description of a scientific approach to clarifying a complicated physiologic feedback system.

Robertson and co-workers have plotted con-

comitant levels of plasma AVP and osmolality in a large number of experimental animals and showed that they were related by the function $\text{plasma AVP} = k (\text{plasma osmolality} - X)$ where k was the slope of the regression line and X was the X intercept (Figure 9).^{103,104} They proposed

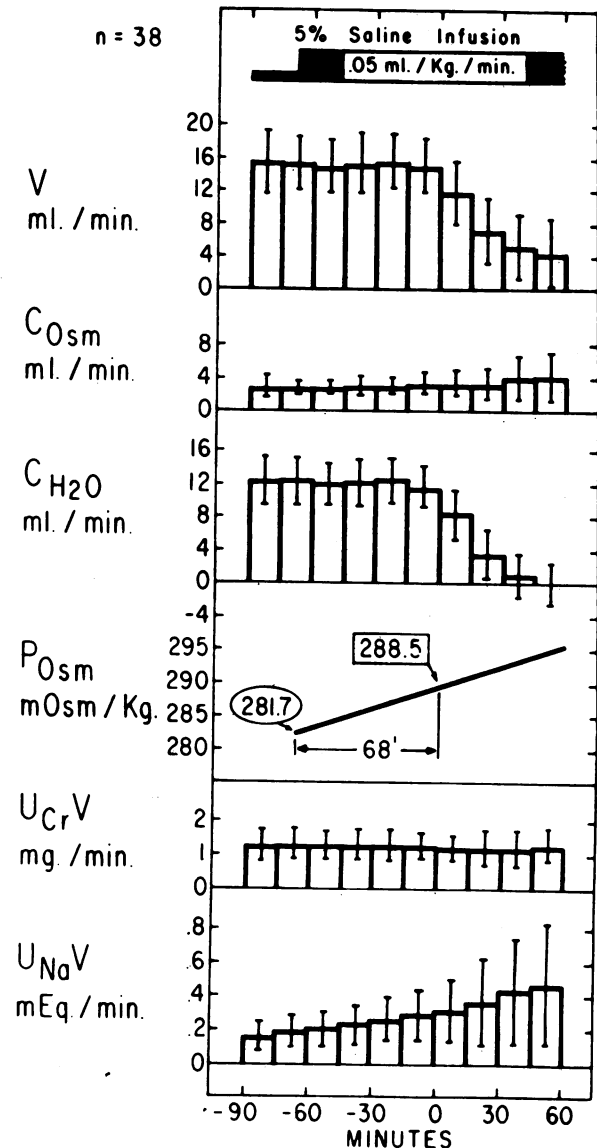


Figure 7.—Composite osmotic threshold studies on 38 normal subjects. Mean osmotic threshold in mOsm per kg of body weight is entered in rectangle and mean plasma osmolality (P_{Osm}) at the beginning of the rapid saline solution infusion is entered in ellipse. Bars and brackets represent mean \pm standard deviation for urinary volume (V), osmolar clearance (C_{Osm}), free water clearance (C_{H_2O}), creatinine excretion (U_{CrV}) and sodium excretion (U_{NaV}). Time intervals are expressed in relation to the time of the osmotic threshold. (Reproduced with permission from Moses AM, et al: *Am J Med* 42: 368-377, 1967.)

that the X intercept represented the "osmotic threshold" which was the critical level of osmolality at which AVP secretion would be stimulated.^{103,104} The threshold concept had been proposed earlier by Moses and colleagues on the basis of the abrupt onset of a decrease in free water clearance in water-loaded subjects undergoing an infusion of hypertonic saline.¹⁰¹

Alternative theories on the osmotic control of AVP secretion have been proposed. Studies in our laboratory have suggested that instead of a discrete level of plasma osmolality above which AVP secretion would be initiated, there was rather a continuous relationship between the logarithm of plasma AVP and plasma osmolality (Figure 10).¹⁰⁵ This type of relationship also appears to exist between log plasma AVP and blood volume after hemorrhage. Several Scandinavian investigators have raised the question of whether a nonspecific alteration in osmolality was the factor responsible for initiation of thirst or AVP release. Eriksson and co-workers showed that intracarotid injection of hypertonic glycerol or galactose failed to produce an antidiuresis comparable to that occurring after injection of hypertonic saline or sucrose.¹⁰⁶ Similarly, injections of hypertonic saline into the lateral ventricles of the brain produced intense

hyperdipsia and antidiuresis, while hypertonic solutions of sucrose, d-glucose or glycerol brought about increased free water clearance.^{107,108} This effect of cerebroventricular infusion of sodium on thirst and antidiuresis could be augmented by concomitant administration of angiotensin II.¹⁰⁷ These results suggested the presence of a central, sodium-sensitive receptor near the third ventricle that was capable of controlling both thirst and AVP secretion. It appeared that angiotensin either enhanced sodium transport to the regulatory structures or that it sensitized them in some fashion. The contradictions between this hypothesis and the more general osmoreceptor hypothesis have not been resolved.

The osmotic stimulation of AVP secretion can be modified significantly by changes in volume of body fluids. Both the apparent osmotic threshold and the sensitivity (slope) of the osmolality-plasma AVP relationship were shown to be altered by modification of extracellular fluid or plasma volume (Figure 11). Hypovolemia in experimental animals resulted in lowering of the osmotic threshold, while the slope of the response relationship became steeper.¹⁰³ In later studies in humans, upright posture was shown to lower the osmotic threshold compared to the recumbent posture.¹⁰⁹

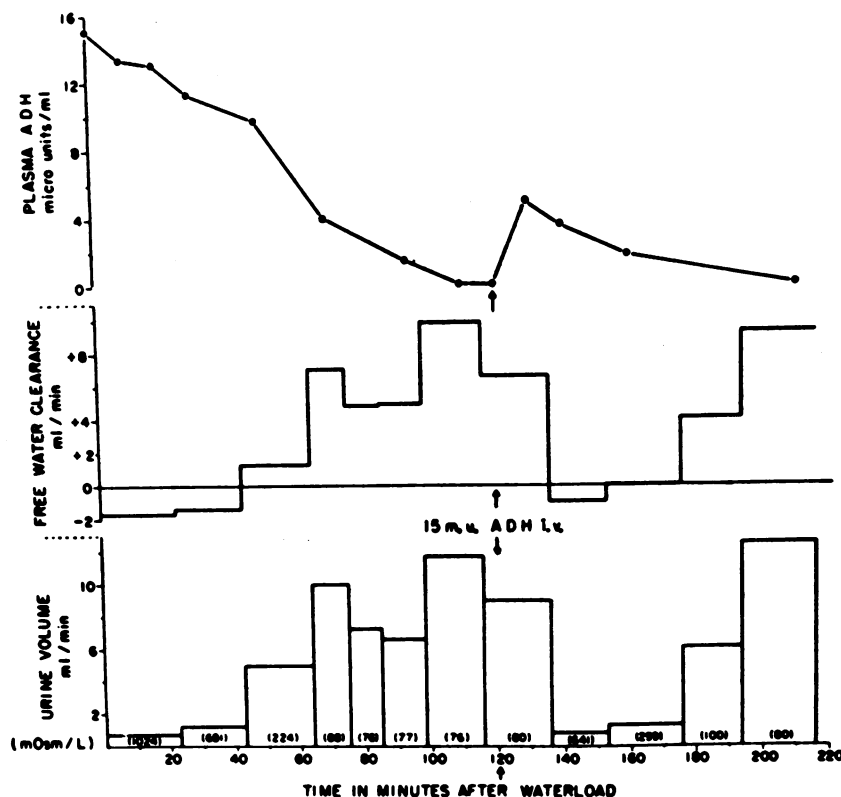


Figure 8.—Effect of a sustained oral water load (2,000 ml), followed by the intravenous injection of arginine vasopressin, on the plasma level of antidiuretic hormone (ADH) and the measurement of a maximal water diuresis. (Reproduced with permission from Czaczkes and Kleeman.¹⁰¹)

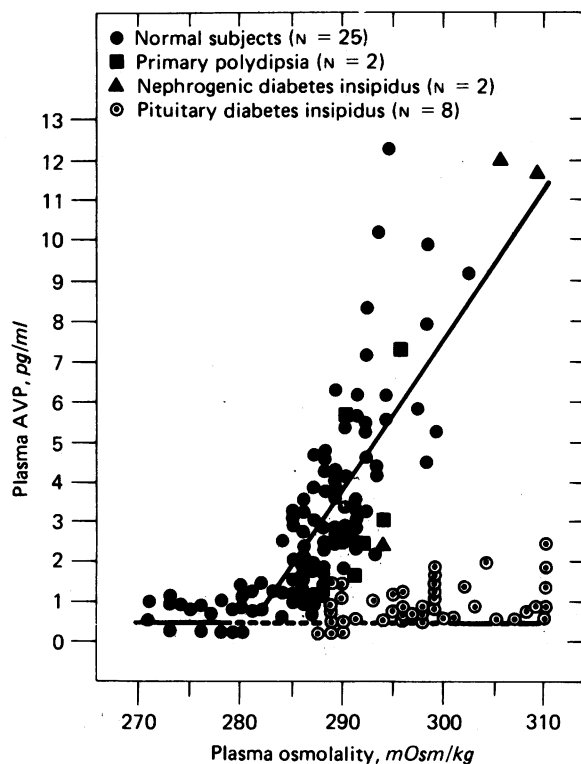


Figure 9.—Plot of plasma arginine vasopressin (AVP) and plasma osmolality in a series of normal subjects as well as in those with primary polydipsia, nephrogenic diabetes insipidus and pituitary diabetes insipidus. The best fit regression line is shown with the solid line. (Reprinted with permission from Robertson GL, Shelton RL, Athar S: The osmoregulation of vasopressin. *Kidney International* 10:25-37, 1976.)

In these studies there was no significant change in the slope. Intravenous infusion of angiotensin II in dogs significantly steepened the slope of the relationship between plasma osmolality and plasma AVP and served to potentiate osmotically stimulated AVP release.¹¹⁰ These observations suggested an interaction of osmolar and volume factors so that AVP secretion at any moment is controlled by the algebraic sum of the stimulating and inhibiting impulses impinging on the final common pathway.

From the above, the importance of volume as well as osmolality of the body fluids in the control of the secretion of AVP is immediately apparent. A change in vascular or extracellular volume, or both, exerts its effect through baroreceptor or pressure receptor mechanisms somewhere in the circulation. In Table 3, under 2a and 2b, these receptors have been listed. In recent years, an increasing number of investigators have studied

the nature of the reflex arcs activated by these receptors.*

These receptors are located in the carotid sinus, the aortic arch, the left atrium and great pulmonary veins and, probably, in the juxtaglomerular apparatus of the kidney.^{16,18,22-24,113} As baroreceptors they respond as tension develops in the wall of the receptor organ rather than to the volume of blood per se; in other words, the higher the compliance (less change in mural tension for any given change in volume), the less will be the stimulation or inhibition of the receptor. The afferent limbs of the first three of these neurohumoral arcs are the ninth or glossopharyngeal nerves from the carotid sinus, the aortic nerves of the aorta, and the tenth or vagus nerve from the wall of the left atrium and great veins. Their impulses ascend from the medulla by way of the reticular formation of the midbrain and diencephalon to the supraoptic nuclei in the hypothalamus. A *decrease* in afferent impulses from these baroreceptors, caused by the stimuli listed in Table 3 brings about an *increase* in the secretion of AVP; conversely, an *increase* in the afferent impulses caused by the inhibitors listed in Table 3 results in a *decrease* of the secretion of AVP.

Section of the thoracic or cervical vagi, or denervation of the carotid sinus, will completely block these nonosmotic stimuli that lead to the release of antidiuretic hormone. Similarly, denervation of the vagus will result in a more exaggerated rise in the circulating level of AVP when a stimulus is applied that lowers pressure in the carotid sinuses as well as in the great veins of the thorax and the left atrium (Figure 12). Conversely, if the pressure in the carotid sinus is maintained despite a fall in cardiac output or blood volume, either the increment will be substantially less or the rise in the circulating level of antidiuretic hormone will be prevented entirely. Share, in his classic studies, has shown that within a few minutes after reduction of blood pressure of dogs to 50 mm of mercury by bleeding, there is a rapid discharge of vasopressin AVP into the blood.^{8,112} Carotid perfusion to maintain a high pulse pressure, in a dog in which a vagotomy had been done prevented this discharge (Figure 13).

Independent of its effect on renal hemodynamics, contraction of the extracellular or plasma volume without an alteration in the tonicity of the body fluids may cause a striking release of AVP. Positive-pressure breathing, which decreases the

*References 8,9-13,16,18,22-24,26,38,111-113

intrathoracic blood volume, causes a significant release of AVP. Similarly, negative-pressure breathing with a rise in intrathoracic volume is followed by a typical water diuresis. This volume receptor, or more correctly baroreceptor, in the left atrium or great pulmonary veins, appears to be extremely sensitive to small shifts in volume or tension.

Alterations in posture have been thought to modify AVP secretion in humans. Moore and co-workers noted a significant elevation in plasma AVP in persons upon assumption of an upright posture.^{11,17} However, the large changes in bioassayable AVP concentration that they observed have not been confirmed in more recent studies using radioimmunoassay. Athar and Robertson were able to show an increase in plasma AVP in persons in an upright position compared with those in a supine position only in dehydrated subjects (Figure 14).¹⁰⁹ They showed that upright posture influenced the relationship between plasma osmolality and plasma AVP by lowering the apparent osmotic threshold. In contrast, upright posture produced by head-up tilting caused

significant elevations of plasma AVP.¹¹⁴ This occurred in a biphasic pattern with a small initial increase after ten minutes of 85 degrees of tilt followed by a considerably larger increase after 45 minutes. The initial hypovolemic stimulus appeared to be due to venous pooling that led to transudation of protein-free fluid through dependent vascular beds and the later decline in plasma volume. Therefore, it appears that posture (the upright position) exerts a tonic nonosmotic stimulus to the sustained neurohypophyseal-released AVP, and, when one reclines, this stimulus is lessened, AVP secretion decreases and a mild diuresis of more dilute urine occurs. Epstein and associates have clearly shown that head-out immersion in humans creates an antigravity model whose cardiovascular (and renal) effects closely simulate those of the reclining position. In one of their studies they determined urinary AVP excretion in ten normal subjects undergoing immersion after 14 hours of overnight water restriction. The immersion resulted in a progressive

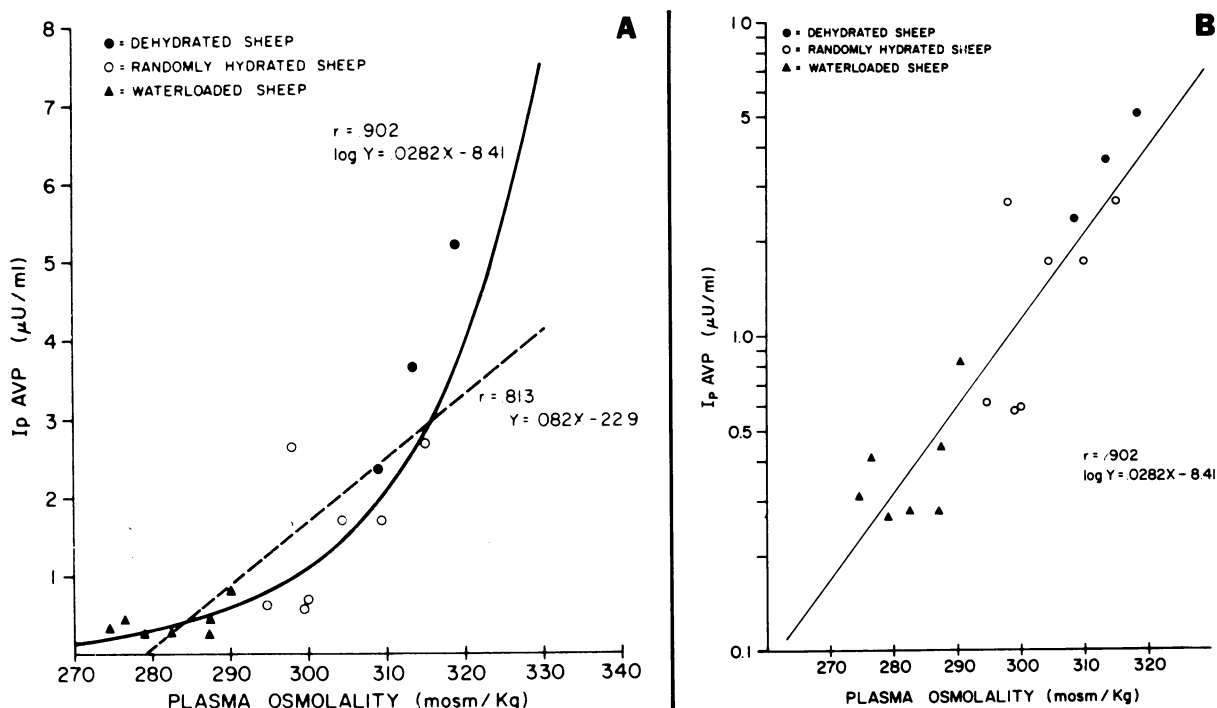


Figure 10.—A, Integrated plasma arginine vasopressin (AVP) plotted as a function of plasma osmolality (OSM) in sheep. Each point represents the mean or integrated plasma AVP concentration determined from ten samples collected at three-minute intervals. The best fit for the equation $AVP = k_1 P_{Osm} + b$ is shown with the dashed line, while the best fit for the equation $\log AVP = k_2 P_{Osm} + b$ is shown with the solid line. The logarithmic plot appears to better fit the data, particularly at the extremes of plasma osmolality. **B**, The data are replotted on a logarithmic scale. The linear relationship between log AVP and plasma osmolality is apparent when plotted in this fashion. (Reproduced with permission from Weitzman R, Fisher DA: Log linear relationship between plasma arginine vasopressin and plasma osmolality. *Am J Physiol* 233:E37-E40, 1977.)

decrease in AVP secretion from 80 ± 7 to 37 ± 6 μU per minute during the recovery hour.¹¹⁵

Zehr, Johnson and Moore¹³ by carrying out detailed studies on normal, unanesthetized sheep, concluded that sensitive osmoreceptors and volume receptors (baroreceptors) exist that are involved in the regulation of AVP secretion. At the stimulus level used in their studies, neither receptor system dominated the other and, under normal circumstances, they act in concert to maintain

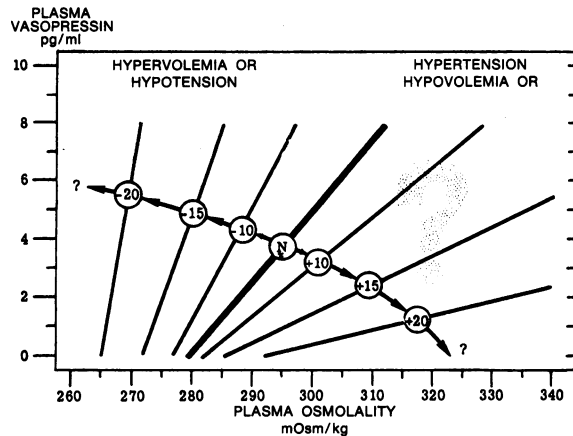
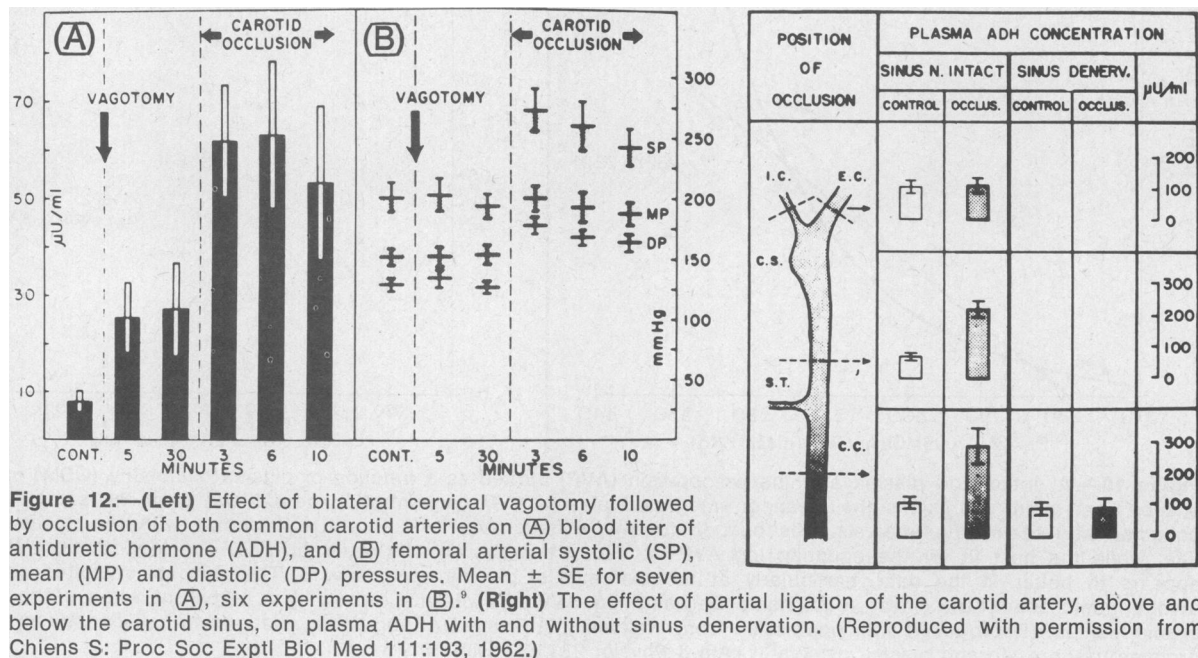


Figure 11.—Schematic diagram of the effect of alterations of blood volume or pressure on the relationship between plasma osmolality and plasma vasopressin. Decreased blood volume or blood pressure appears to enhance vasopressin release at any particular level of plasma osmolality, while increased blood volume has the opposite effect. (Reproduced with permission from Robertson GL, Shelton RL, Athar S: The osmoregulation of vasopressin. *Kidney International* 10:25-37, 1976.)

the osmolality and volume of the extracellular fluid via their effect on AVP secretion. It is of great interest that those changes in the circulation and the volume and distribution of body fluids that stimulate the baroreceptors of the carotid sinus and left atrium (Table 3) are also the changes that bring about the release of renin from the kidneys, and thereby the production of angiotensin. If angiotension, through its direct action on hypothalamic centers, can not only stimulate thirst but can also augment the release of AVP,^{16,18,22-24,113} then we must consider an additional regulatory system contributing to the modulating impulses impinging on the final common pathway, the supraoptic neurons. The tonic nature of the stimuli arising from the upright position can be seen readily by observing the increase in flow of a more hypotonic urine that almost invariably follows the assumption of a reclining position. From this, one would expect a similar diuresis to develop after the subject has retired for the night. However, this is not observed and, on the contrary, the sleeping hours are, fortunately, accompanied by a period of antidiuresis associated with an elevated plasma concentration of AVP. The mechanism responsible for the nocturnal antidiuresis obviously overrides the inhibitory stimulus of the reclining position as such. This nocturnal antidiuresis is only explained in part by the nocturnal decrease in glomerular filtration rate and solute excretion.



Many disease states, particularly those associated with a tendency toward sodium and water retention (such as cirrhosis, cardiac failure, nutritional edema and nephrosis) are almost invariably associated with a reversal of the normal diurnal pattern of water excretion. These disorders, accompanied by an alteration or redistribution of the vascular volume, also exaggerate the postural effect on the release of AVP. Therefore,

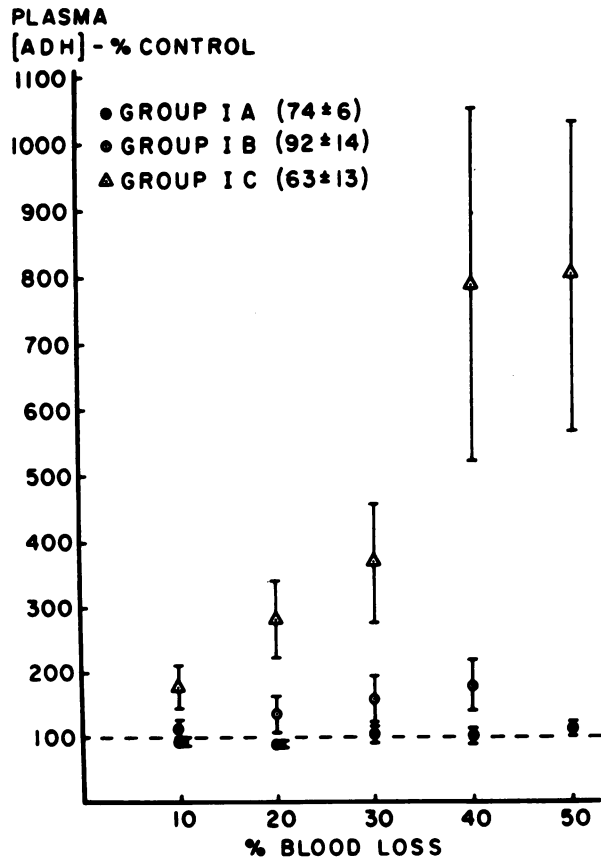


Figure 13.—Effect of hemorrhage on the concentration of antidiuretic hormone (ADH) in plasma, when the carotid sinuses were perfused with a high pulse pressure. The concentration of ADH in the first plasma sample of each experiment is taken as 100 percent. The ADH concentrations in subsequent samples are expressed as percent of the concentration in this first sample. These values are indicated on the ordinate. The abscissa is the degree of blood loss, expressed as percent of the initial blood volume, which was estimated to be 8 percent of the body weight. The lines extending above and below the symbols indicate \pm SE (standard error). The figures in parentheses are the concentrations of ADH in the initial blood samples in microunits per milliliter plasma (mean \pm SE). There were six experiments in group 1A, eight in group 1B, and eight in group 1C. Group 1A, control group; group 1B, perfused, with high pulse pressure during hemorrhage; group 1C, hemorrhage. (Reproduced with permission from Share.¹¹²)

it is probable that while exercising or in the upright position, these patients, despite their expanded extracellular volume, have a far greater release of AVP than do normal subjects. The assumption of the reclining position during the night may actually decrease the amount of AVP and lead to a nocturnal water diuresis. This is suggested in the classic studies of Borst and de Vries.¹¹⁶ It is probable that the improved renal hemodynamics, reduced secretion of aldosterone and AVP, and increased circulation of the natriuretic factor^{117,118} all contribute to the nocturnal polyuria that is so characteristic of the disease states mentioned above. That this reversal is also seen in states of primary or secondary adrenal insufficiency suggests a role of the glucocorticoids in the maintenance of the normal diurnal pattern. However, no clear-cut relationship has been established. One might ask why in the edematous states mentioned earlier (especially those such as con-

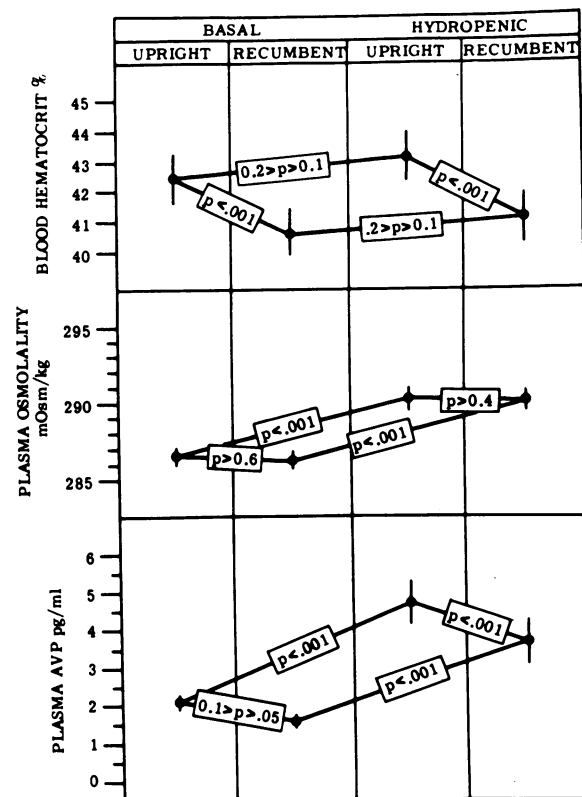


Figure 14.—The effect of dehydration and upright posture on plasma arginine vasopressin (AVP), osmolality and hematocrit in humans. A significant rise in plasma AVP in the upright posture compared with the recumbent posture was seen only when the subjects were hydropenic. (Reproduced with permission from Robertson GL, Shelton R, Athar S: The osmoregulation of vasopressin. *Kidney International* 10:25-37, 1976.)

gestive heart failure and cirrhosis in which blood volume is also increased) we do not see a raised osmotic threshold for AVP release as described in volume-expanded normal humans¹⁰¹ and animals.¹⁰² The most likely explanation is that impaired circulation or redistribution of blood, or both, seen in those states produce an *abnormal* response by the baroreceptors of the carotid sinus, left atrium and juxtaglomerular apparatus. Strong support for this explanation comes from the study of decompensated cirrhosis in patients by Epstein and associates using water immersion to the neck, a procedure that redistributes blood volume with concomitant central hypervolemia. This model assessed the role of *effective volume* in the impairment of sodium and water handling in patients with cirrhosis. They observed a striking rise in free water clearance (C_{H_2O}) and drop in minimal urinary osmolality (Figures 15 and 16) which were most consistent with the interpretation that both avid reabsorption of filtrate proximal to the diluting site and increased levels of AVP may participate to varying degrees in mediating the impaired water excretion accompanying cirrhosis.

It is well recognized that during very rigid sodium restriction in normal persons, the early negative salt balance during the first few days, before there is maximal renal conservation of sodium, is associated with a weight loss ranging from 0.5 to 2.0 kg. During this period, water ingested is readily excreted in isotonic proportions with salt. Further, negative salt balance and loss of extracellular volume may substantially impair water diuresis and urinary dilution. This was clearly shown in the early experiments in humans by McCance¹¹⁹ and in studies in dogs and humans by Leaf and his associates (Figure 17).^{120,121} Studies in our laboratory have shown that mild salt restriction for three days in normal subjects produced a statistically insignificant rise in plasma AVP. At the same time there was a significant fall in plasma tonicity.¹²² In this setting, AVP secretion was inappropriate for the concomitant level of plasma osmolality. Under these conditions, volume factors were responsible for the relative elevation of plasma AVP. There is no doubt that the reduction in renal blood flow and glomerular filtration rate, the pronounced conservation of sodium and the sustained nonosmotic release of AVP all contribute to the severity of the impaired water diuresis. In many disorders associated with extensive loss of vascular or extracellular volume, the nonosmotic stimulus to the release of AVP

may be of such magnitude and duration that it may override the inhibitory effect of hypo-osmolality and cellular overhydration. When this occurs, hypotonic retention of water and hyponatremia invariably follow. Although this might be considered an *inappropriate* secretion of AVP, it

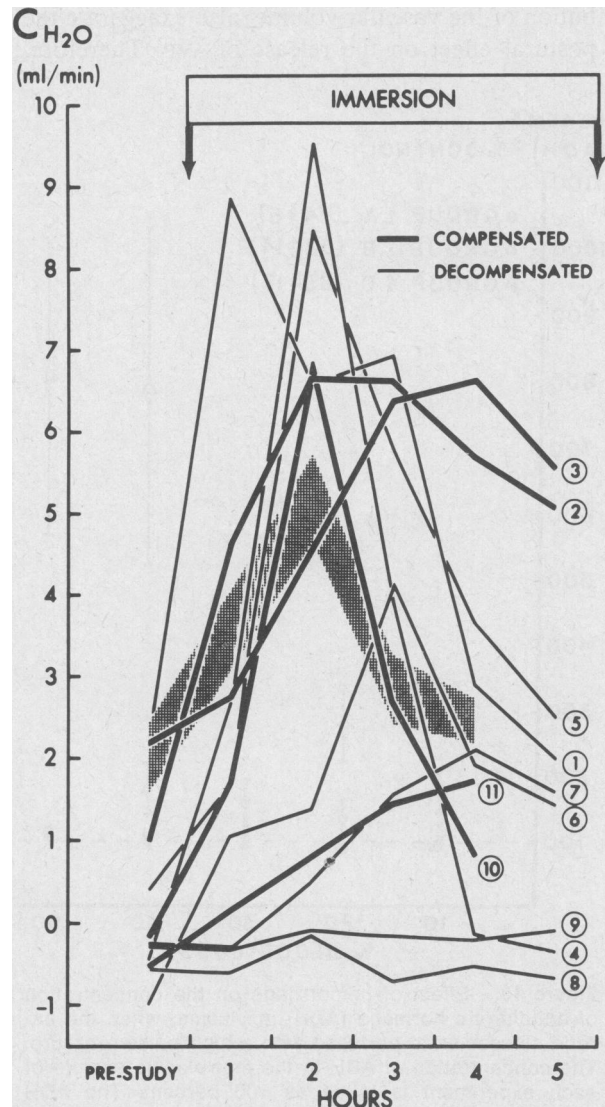


Figure 15.—Effects of water immersion following one hour of quiet sitting (prestudy) on free water clearance (C_{H_2O}) in 11 patients with alcoholic liver disease. Shaded area represents the mean \pm SE for clearance for 18 normal control subjects undergoing immersion while in balance on a 150 mEq sodium diet. These controls received a water load (1,700 ml) which was twice as great as that used in the present study. Immersion resulted in a significant increase in C_{H_2O} ($P=0.01$) which equalled or exceeded that of the controls on the higher sodium intake. (Reproduced with permission from Epstein M, et al: Determinants of deranged sodium and water homeostasis in decompensated cirrhosis. *J Lab Clin Med* 87:832, 1976.)

is apparent that the body is merely sacrificing a small change in tonicity to buffer a larger change in volume.

Any major surgical operation, with its accompanying premedication, anesthesia, incisions, traction on viscera, decrease in blood volume and cardiac output and, finally, postoperative pain, creates a constellation of nonosmotic stimuli which lead to sustained operative and postoperative release of AVP (Figure 18). This, more than any reduction in renal blood flow or nonhormonal

impairment in water excretion, is responsible for the relative or absolute impairment in water excretion in the postoperative period and the very common symptomatic or asymptomatic hyponatremia observed so frequently following surgical procedures.

From our earlier discussion on thirst, it was evident that hypothalamic centers regulating the thirst sensation could be stimulated by impulses from the cerebral cortex. This also holds true for the release of AVP. It is well known that pain can bring about a sudden release of AVP and antidiuresis and that inappropriate antidiuresis has been observed in psychotic patients. Demerol (meperidine hydrochloride), barbiturates and morphine, all capable of stimulating AVP release when given in large amounts, may act through the baroreceptor mechanisms (Table 3) rather than by a direct pharmacologic effect on the neurohypophyseal system.

In an earlier section, we discussed the effect of various pharmacologic agents (chemicals) on the regulation of thirst and their role as possible neurohumoral transmitters in the hypothalamus. These drugs, or their analogues, also alter the release of AVP or the activity of the supraoptic neurons.¹²³⁻¹²⁵ The cholinergic agents, acetylcholine, mecholyl or carbachol, when injected systemically or into the carotid artery, or applied locally to the supraoptic nuclear area, stimulate the immediate release of AVP. It is of great interest that these drugs cause a water repletion reaction, and inhibit food ingestion simultaneously.³¹ Ingestion of food when water must be conserved would tend to enhance solute and water excretion. Possibly acting through these neurochemical transmitters are the effects created by warm blood perfusing the hypothalamus. Overheating of an animal or direct heating of the hypothalamus creates a sensation of thirst, a stimulation leading to the release of AVP, antidiuresis and inhibition of food intake, the water repletion reaction. However, the sensation of thirst and the release of AVP after exposure to a hot environment may also be due to stimulation of the baroreceptor mechanisms listed in Table 3. The peripheral vasodilatation and the associated redistribution of blood, with a decrease in central blood volume could stimulate the receptors. This is the interpretation of Segar and Moore,¹² who found that exposure of normal subjects for two hours at 50°C caused a four- to fivefold increase in plasma AVP. The critical importance of this

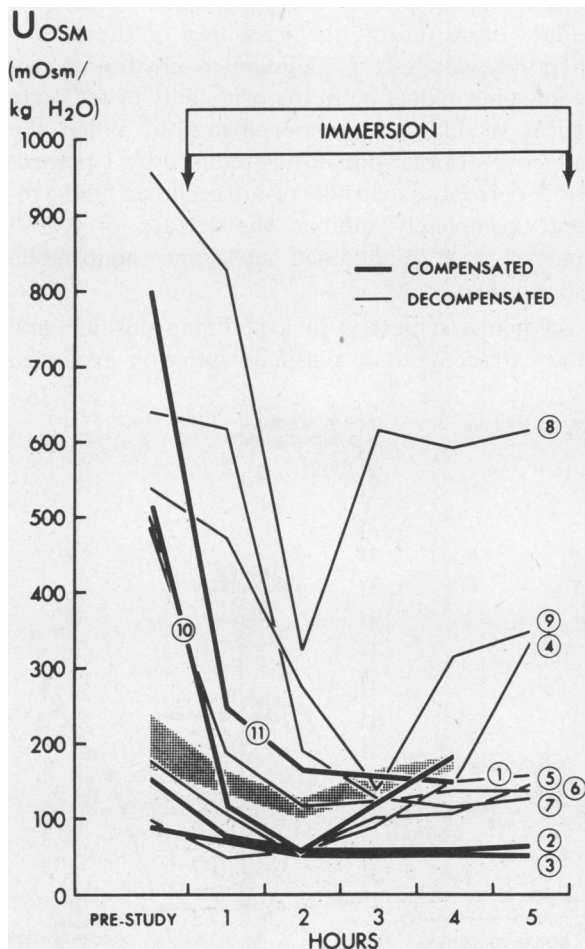


Figure 16.—Effect of water immersion following an hour of quiet sitting (prestudy) on total urinary solute concentration (U_{Osm}) in 11 patients with alcoholic liver disease. Shaded area represents the mean \pm SE for U_{Osm} for 18 normal subjects undergoing immersion. These controls received a water load twice as great as that used in the present study. The urine was substantially diluted in all the patients, attaining levels of minimal U_{Osm} equal to or less than that of the controls. (Reproduced with permission from Epstein M, et al: Determinants of deranged sodium and water homeostasis in decompensated cirrhosis. *J Lab Clin Med* 87: 833, 1976.)

water repletion reaction to persons in a hot environment is obvious.

Recent studies have indicated that the beta-adrenergic agent isoproterenol (Isuprel), when infused systemically into normal human volunteers, anesthetized¹²⁶ and trained unanesthetized dogs,¹²⁷ all undergoing a maximal sustained water diuresis, and into rats with congenital diabetes insipidus,¹²⁸ caused an antidiuresis that was indistinguishable from that caused by AVP. However, a direct renal arterial injection of isoproterenol had no antidiuretic effect.^{126,129} It is still unclear whether this beta-adrenergic antidiuresis was due primarily to the release of AVP, or to the circulatory effects of the drug, or both. The antidiuresis can be completely inhibited by the alpha-adrenergic agent norepinephrine.¹²⁷ The latter inhibits the action of antidiuretic hormone at the renal tubule^{127,130} and appears to inhibit the electrical activity of the supraoptic neurons¹²³ and the release of AVP as well. Norepinephrine also suppresses AVP release via elevation of blood pressure and stimulation of baroreceptors. These studies on the interrelationship of catecholamines

with AVP and water metabolism make it necessary, as with the renin-angiotensin system, to consider still another set of neurohumoral regulators which may influence the hypothalamic centers controlling thirst and the release of AVP.

Osmotic and Nonosmotic Inhibitors of AVP Secretion

The physiologic inhibitors of the release of AVP (Table 3) involve a reduction in intracellular osmolality (cellular dehydration), and an expansion of the plasma or extracellular fluid volume. Water diuresis with the production of a very dilute urine reflects the inhibition of the neurohypophyseal system. Cellular overhydration accompanies reduction in the osmolality of the body fluids secondary to water ingestion. When the effective osmotic pressure is reduced by 1 percent or 2 percent, expansion of intracellular fluid volume completely inhibits the release of AVP¹³¹ unless it is maintained by some nonosmotic stimulus.

When a subject is in a reclining position and the extracellular or plasma volume is expanded

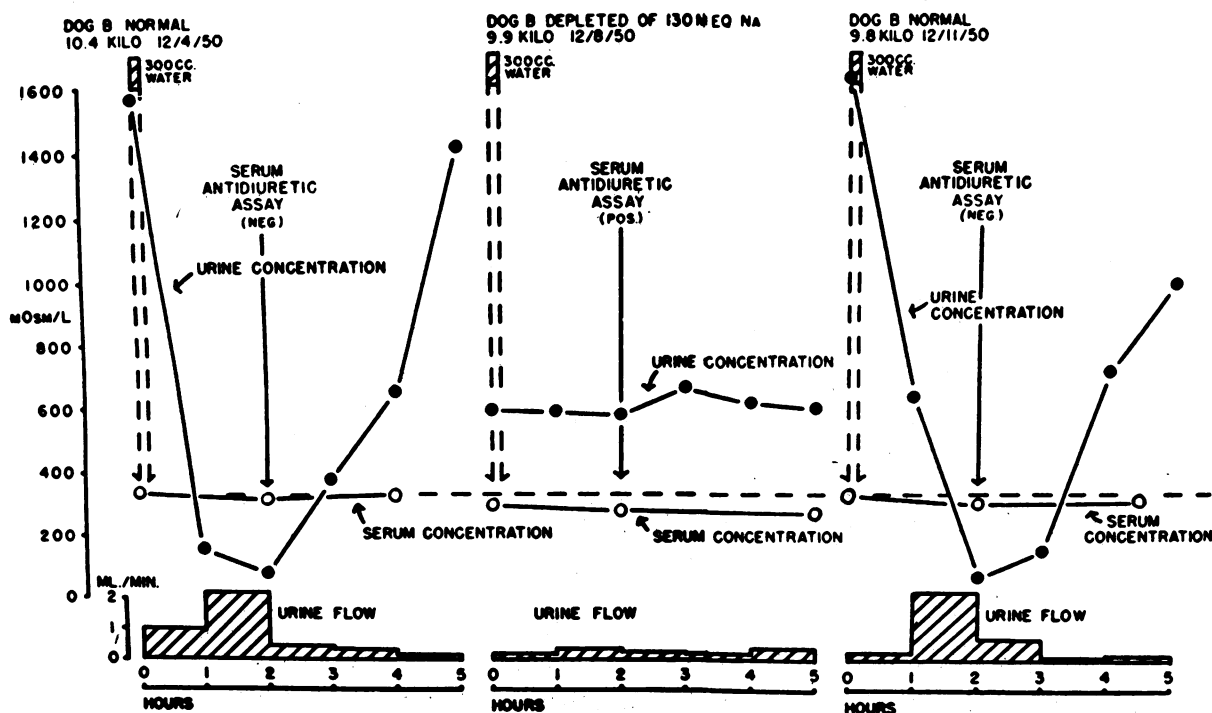


Figure 17.—The right- and left-hand portions show the diuretic pattern before and following depletion. These are entirely normal diuretic patterns. The middle section shows conversion to the abnormal antidiuretic pattern following removal of 130 mEq sodium by peritoneal dialysis. In this section, one can note a dilute serum but a hypertonic urine and failure of dilution of the urine or diuresis following water administration. Antidiuretic activity was observed in the serum during depletion but not in the controls. (Reproduced with permission from Leaf A, Mamby AR.¹²⁰)

with such solutions as isotonic saline or iso-oncotic albumin, a characteristic water diuresis occurs. These solutions, although expanding the plasma and extracellular volume, do not alter intracellular volume or the osmolality of the body fluids. It is, therefore, apparent that volume expansion as such can influence the baroreceptor impulses from the carotid sinus and left atrium (Table 3) and inhibit the renin-angiotensin system, thereby inhibiting the release of AVP.

We mentioned earlier the experiments by Moses and associates^{100,101} in humans and Zehr and co-workers¹³ in the unanesthetized sheep, both clearly showing that the osmotic release of AVP can be substantially decreased by previous isotonic expansion of plasma or extracellular volume or both. Further, when 400 to 500 ml of hypertonic saline (5 percent) is administered to a subject who is under the influence of a sustained positive water load (1,300 to 1,500 ml), a typical antidiuresis may not develop in spite of a pronounced hyperosmolality that causes withdrawal of water from the cells. This is due to the fact that the further expansion of the extracellular volume, which occurs in these subjects after the hypertonic salt administration, is great enough to blunt the stimulatory effect of the hyperosmolality and, therefore, decreases the release of AVP; also, on occasion, the increased excretion of sodium and osmolar clearance may be so great that despite a substantial decrease in free water clear-

ance, indicating an approximate inhibition of the water diuresis, the rate of urine flow may not decrease.

Nonosmotic stimulation of the baroreceptors, and their inhibition of AVP release, may be produced clinically by an episode of paroxysmal atrial arrhythmia in a person with a basically normal heart. This will cause a sudden increase in cardiac output and, possibly, left atrial tension. The inhibition of AVP secretion causes a water diuresis.¹³² The polyuric syndrome that may accompany sudden cardiac arrhythmias which do not significantly impair myocardial function has been known for years. We have had the opportunity to study one such polyuric patient who, despite moderate dehydration and a rise in plasma osmolality of approximately 20 percent, excreted a persistently hypotonic urine; no AVP could be detected in his plasma.

This case is an example of altered osmotic release of AVP caused by stimulation of the cardiac baroreceptors which, in turn, led to a nonosmotic inhibition of AVP secretion and hyperosmolality (hypernatremia). This is analogous to the effect of isotonic plasma or extracellular volume expansion on the osmotic threshold.^{13,102} From these clinical and experimental observations it is possible to explain the mild hyperosmolality and hypernatremia seen in many patients with primary hyperaldosteronism. The excess mineralocorticoid secretion causes sodium retention and sustained,

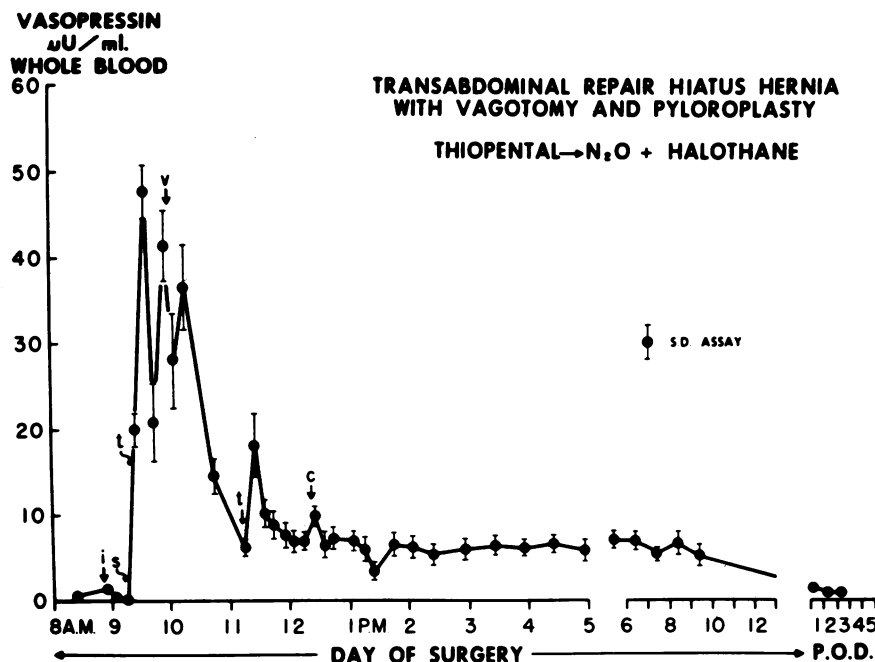


Figure 18.—Changes in blood levels of arginine vasopressin during and after a major surgical procedure. (Reproduced with permission from Zimmermann B: Surg Clin N Am 45: 310, 1965.)

steady-state expansion of the plasma and extracellular volume. The latter elevates the osmotic threshold, and hypernatremia must be present to allow the osmotic release of AVP. The hypernatremia may lead to thirst, a fairly common symptom in these patients; but when water is drunk and the plasma hyperosmolality decreases slightly, AVP secretion is again inhibited and a water diuresis ensues; thus, the hypernatremia is sustained. Such suppression of plasma AVP has been confirmed experimentally in our laboratory.¹²² The administration of 9 α fluohydrocortisone (Florinef) to normal volunteers produced a significant fall in plasma AVP.

The converse of the water repletion reaction which follows the administration of heat, cholinergic and beta-adrenergic agents, is the water depletion reaction, which accompanies the local hypothalamic application, or intracarotid or systemic injection of anticholinergic or alpha-adrenergic drugs such as norepinephrine. The latter may inhibit thirst and the release of AVP,¹²³ stimulate food intake and antagonize the action of AVP

on the distal nephron.^{127,130} As would be expected, exposure of an animal to cold or cooling of the blood perfusing the hypothalamus will cause a decrease in thirst, a water diuresis (the classic "cold" diuresis) and an increase in food intake. Segar and Moore¹² found that exposure of normal subjects to a temperature of 13°C for an hour caused a definite inhibition of AVP release and a fall in its concentration in the plasma. They concluded that this was due to a rise in central blood volume and blood pressure stimulating the left atrial and carotid baroreceptors.

It has been frequently shown that ethyl alcohol is a potent chemical inhibitor of the release of AVP.⁹ This inhibition occurs during a rising level of alcohol in the blood after its oral or intravenous administration, although in humans, at least, the maintenance of a constant blood level does not assure the continued inhibition of AVP release. Alcohol appears to block stimuli that would ordinarily cause the release of AVP (such as hypertonic saline, reduction in blood volume or prolonged venous congestion of the lower extremities).

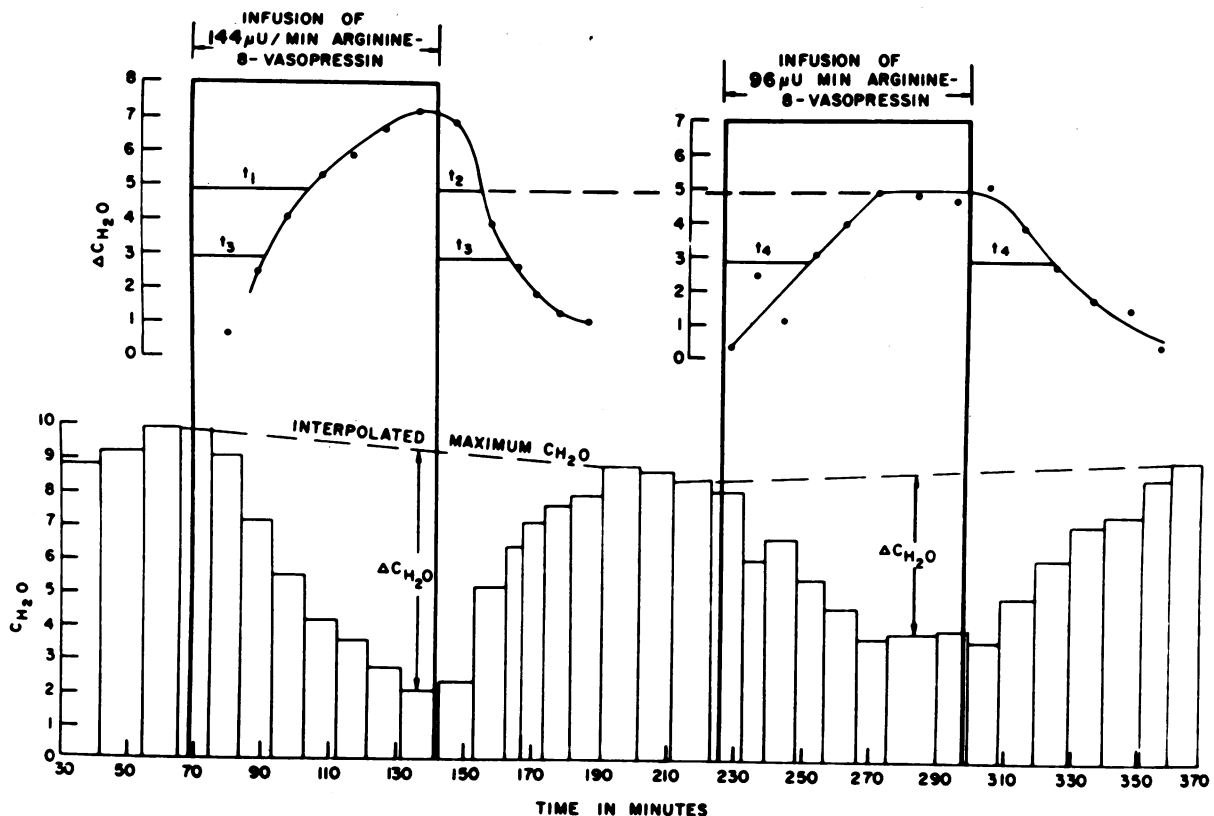


Figure 19.—A typical experiment with infusions of 144 and 96 μU per minute arginine-8-vasopressin. The C_{H_2O} is graphed for each period. The change in C_{H_2O} (ΔC_{H_2O}) is determined by the difference between the interpolated maximum C_{H_2O} and the actual C_{H_2O} for each period. (Reproduced with permission from Miller L, et al: Relative potency of arginine-8-vasopressin and lysine-8-vasopressin in humans. J Lab Clin Med 69:270-291, 1967.)

Therefore, the water diuresis that frequently follows the administration of ethyl alcohol, or the ingestion of one or two cocktails, probably represents the inhibition of certain tonic stimuli continuously reaching the neurohypophyseal nuclei. Alcohol has been used in certain clinical states (such as cirrhosis and congestive heart failure) to determine whether the observed oliguria and hypertonic urine are due to continued release of AVP. Usually, between 1 and 3 ounces of 100 proof whiskey, or its equivalent, is necessary for maximal temporary inhibition of AVP release. Unfortunately, alcohol in these amounts has not proved to be especially useful in investigation of most states associated with inappropriate or sustained nonosmotic release of AVP. Recently, diphenylhydantoin (Dilantin) has also been shown to inhibit the release of AVP in humans when administered intravenously.¹³³ This inhibition, as with alcohol, is quite transient and nonsustained. This also limits its usefulness in clinical states associated with inappropriate release of AVP.

Metabolism of AVP

AVP turnover can also be assessed by determining the biologic half-life of AVP *in vivo*. Such determinations have given a value of 16 to 20 minutes for the half-life of AVP.^{94,95} AVP is infused intravenously into subjects undergoing a maximally sustained water diuresis. This will cause a reproducible degree of antidiuresis in a given patient under carefully controlled conditions. When the infusion is stopped, the rate of return of the urine flow to its maximal level reflects the rate of disappearance of the hormone from the circulation. An example of this is shown in Figure 19. The half-time calculated by this indirect technique⁹⁵ correlates quite well with that determined by direct bioassay of the hormone in the circulation,^{94,96} but is somewhat slower than those determined by immunoassay in the separate studies cited above. Once secreted, AVP is rapidly cleared from the circulation. In humans, the plasma half-life of AVP, estimated by radioimmunoassay after pulse injection or after termination of a constant infusion of hormone, ranges from six to nine minutes.¹³⁴⁻¹³⁶ Plasma disappearance follows a biexponential pattern with a rapid first phase and somewhat slower second phase (Figure 20). AVP is cleared in approximately equal fractions by the kidneys and liver.⁹⁶ Renal clearance persists after ureteral ligation and, thus, appears to take place largely in the postglomerular circulation.¹³⁷ Total

clearance declines as the plasma concentration of AVP rises above the physiologic range.¹³⁸ These and other data have suggested a role of high-affinity plasma membrane receptors in mediating AVP clearance.¹³⁹ It is postulated that the receptors bind the biologically active hormone and facilitate its transfer from the vascular compartment to an extravascular compartment where enzymatic degradation occurs.¹³⁹

AVP can be degraded directly in plasma at a rate of 11 percent per hour,¹⁴⁰ but this is relatively

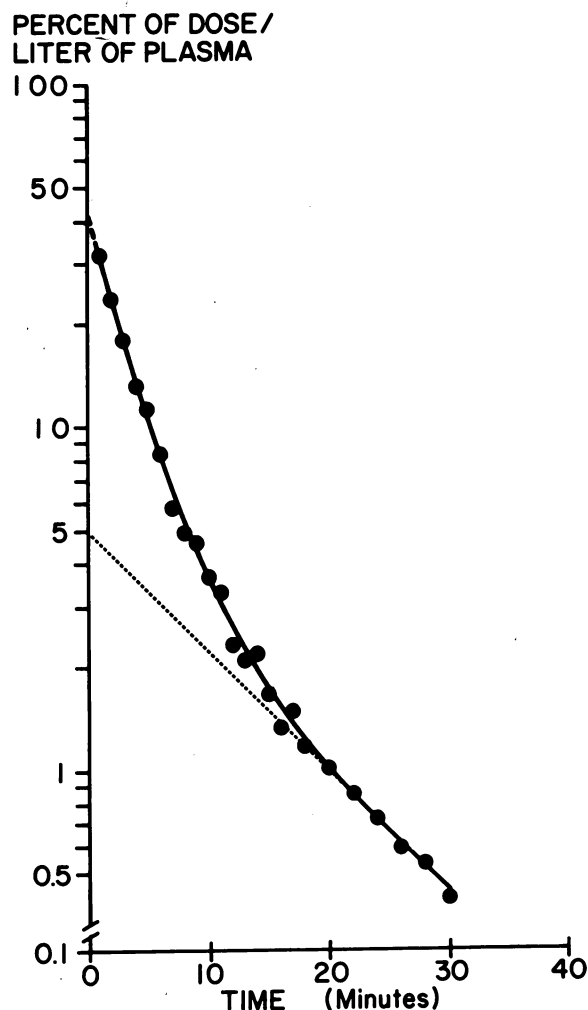


Figure 20.—Plasma arginine vasopressin measured by radioimmunoassay after pulse injection of unlabeled hormone. Two exponentials can be identified. The metabolic clearance rate (MCR) is calculated as the area under the curve and the volume of distribution as the reciprocal of the fractional dose per liter extrapolated to the time of injection. The MCR for six dogs studied in this fashion was $35.5 \pm (\text{SEM}) 0.6$ ml per kg of body weight per minute and the volume of distribution was 12.7 ± 0.9 percent of body weight. (From Weitzman R, Fisher DA: Unpublished data.)

insignificant compared to total body clearance. It is sufficiently great to warrant special handling of plasma samples for AVP assay. During pregnancy in humans and other primates, the placenta produces a circulating enzyme, cystine aminopeptidase (vasopressinase), which considerably augments plasma degradation. The impact of this enzyme on overall hormonal clearance is not known.

Bioassays for AVP have, for many years, been of great value in experimental and clinical studies of normal and abnormal physiology of AVP. However, they are very time consuming and lack the sensitivity of the newer and more specific radioimmunoassays.^{184-187,141} The latter have permitted detection of as little as 0.2 to 0.5 units per ml of plasma. In either radioimmunoassays or bioassays, it is necessary to extract AVP from plasma to separate out substances in plasma that interfere nonspecifically with the assay. In some bioassays, it is necessary to use chemical tests such as inactivation by thioglycolate or pregnancy plasma to exclude the presence of other substances that could evoke either a pressor or antidiuretic response. In the few studies where both types of assays have been used, they generally correlate well; however, a radioimmunoassay may give higher readings due to the presence of immunologically but not biologically reactive hormone fragments. Such fragments may lead to an artifactual overestimation of AVP concentrations by immunoassay compared with bioassay.

Some laboratories express their data in micro-units per ml, using bioassayed USP posterior pitui-

tary extract as a standard. This material is widely available and quite stable so that results from different laboratories may easily be compared. Other laboratories use synthetic AVP as a standard and express their results in picograms per ml. Unfortunately, the potency of commercially available synthetic AVP varies greatly, as determined by bioassay, so that there may be considerable variation in the normal range in different laboratories. One picogram per ml is roughly equivalent to 0.4 units per ml.

Blood specimens for AVP measurement must be quickly chilled after collection and the plasma rapidly frozen to minimize enzymatic degradation. Even so, progressive loss of immunoreactive AVP content of plasma can be observed with prolonged freezer storage. Extraction prevents further degradation.

AVP, like many other hormones, is secreted episodically (Figure 21).¹⁴² Thus, a solitary sample may not accurately reflect neurohypophyseal secretion in a given patient. Collection of several specimens, with pooling of the plasma before assay, has been suggested to minimize such variation.

REFERENCES

1. Kerpel-Fronius E: Clinical consequences of the water and electrolyte metabolism peculiar to infancy, *In* Ciba Foundation Colloquia on Ageing, Vol 4, Boston, Little, Brown and Company, 1958, pp 154-162.
2. Schmidt-Nielsen B: The resourcefulness of nature in physiological adaptation to the environment. *Physiologist* 1:4-20, 1958
3. Wayner MJ: Thirst—First International Symposium on Thirst in the Regulation of Body Water. New York, The Macmillan Company, 1964
4. Andersson B: Polydipsia caused by intrahypothalamic injections of hypertonic NaCl solutions. *Experimentia* 8:157, 1952
5. Andersson B, McCann SM: The effects of hypothalamic lesions on the water intake of the dog. *Acta Physiol Scand* 35: 312-320, 1965

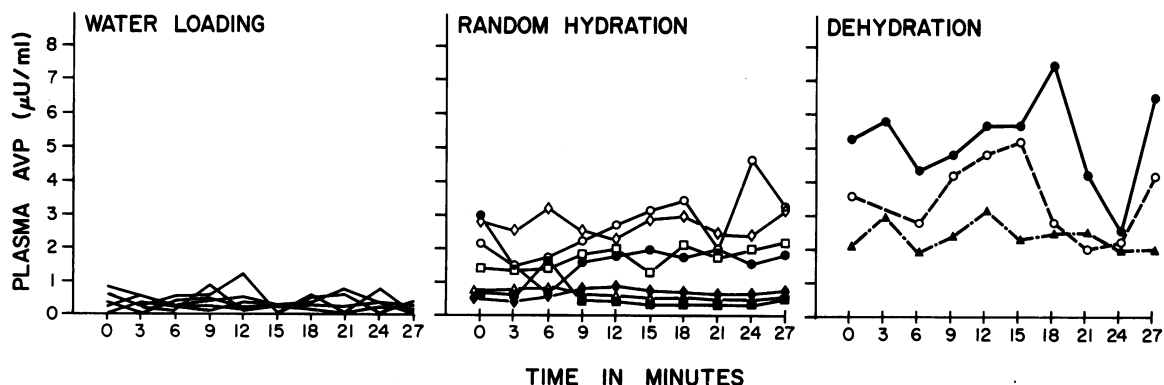


Figure 21.—Plasma arginine vasopressin (AVP) concentration sampled every three minutes for half an hour in sheep. The panel on the left shows only minimal variation in sequential samples collected in water-loaded sheep with low overall mean plasma AVP concentration. Mean AVP levels are higher in the randomly hydrated sheep (center panel) and higher still in the dehydrated animals (panel on the right). A tendency for pulsatile secretion can be observed in some of the randomly hydrated sheep and in the dehydrated sheep. (Reproduced with permission from Weitzman RE, Kleeman CR: Water metabolism and neurohypophyseal hormones, *In* Bondy P, Rosenberg L (Eds): *Diseases of Metabolism*. Philadelphia, WB Saunders, 1979, in press.)

CLINICAL PHYSIOLOGY OF WATER METABOLISM—PART I

6. Stricker EM: Behavioral control of intravascular fluid volume: Thirst and sodium appetite. *Ann NY Acad Sci* 157:553-568, 1969
7. Stricker EM: Osmoregulation and volume regulation in rats: Inhibition of hypovolemic thirst by water. *Am J Physiol* 217:98-105, 1969
8. Share L: Vasopressin—Its bioassay and the physiological control of its release. *Am J Med* 42:701-712, 1967
9. Kleeman CR, Cutler R: The neurohypophysis. *Ann Rev Physiol* 25:385-432, 1963
10. Nash FD: Introductory remarks—Control of antidiuretic hormone secretion. *Fed Proc* 30:1376-1377, 1971
11. Moore WW: Antidiuretic hormone levels in normal subjects. *Fed Proc* 30:1387-1394, 1971
12. Segar WE, Moore WW: The regulation of antidiuretic hormone release in man—I. Effects of change in position and ambient temperature on blood ADH levels. *J Clin Invest* 47:2143-2151, 1968
13. Zehr JE, Johnson JA, Moore WW: Left atrial pressure, plasma osmolality, and ADH levels in the unanesthetized ewe. *Am J Physiol* 217:1672-1680, 1969
14. Fitzsimons JT, Simons BJ: The effect on drinking in the rat of intravenous infusion of angiotensin, given alone or in combination with other stimuli of thirst. *J Physiol* 203:45-57, 1969
15. Epstein AN, Fitzsimons JT, Rools BJ: Drinking induced by injection of angiotensin into the brain of the rat. *J Physiol* 210:457-474, 1970
16. Andersson B, Eriksson L, Oltner R: Further evidence for angiotensin-sodium interaction in central control of fluid balance. *Life Sci* 9:1091-1096, 1970
17. Fitzsimons JT: The effect on drinking of peptide precursors and of shorter-chain peptide fragments of angiotensin II injected into the rat's diencephalon. *J Physiol* 214:295-303, 1971
18. Andersson B, Eriksson L: Conjoint action of sodium and angiotensin on brain mechanisms controlling water and salt balance. *Acta Physiol Scand* 81:18-29, 1971
19. Fitzsimons JT: The role of a renal thirst factor in drinking induced by extracellular stimuli. *J Physiol* 201:349-368, 1969
20. Severs WB, Severs AED: Effects of angiotensin on the central nervous system. *Pharmacol Rev* 415:448, 1973
21. Reid IA, Ramsay DJ: The effects of intracerebroventricular administration of renin on drinking and blood pressure. *Endocrinology* 97:536, 1975
22. Mouw D, Bonjour J-P, Malvin RL, et al: Central action of angiotensin in stimulating ADH release. *Am J Physiol* 220:239-242, 1971
23. Bonjour J-P, Malvin RL: Stimulation of ADH release by the renin-angiotensin system. *Am J Physiol* 218:1555-1559, 1970
24. Nicoll RA, Barker JL: Excitation of supraoptic neurosecretory cells by angiotensin II. *Nature (New Biol)* 233:172-173, 1971
25. Bonjour J-P, Malvin RL: Plasma concentrations of ADH in conscious and anesthetized dogs. *Am J Physiol* 218:1128-1132, 1970
26. Malvin RL: Possible role of the renin-angiotensin system in the regulation of antidiuretic hormone secretion. *Fed Proc* 30:1383-1386, 1971
27. Keil LC, Summy-Long J, Severs WB: Release of vasopressin by angiotensin II. *Endocrinology* 96:1063-1065, 1975
28. Sirois P, Gagnon DJ: Increase in cyclic AMP levels and vasopressin release in response to angiotensin I in neurohypophyses: Blockade following inhibition of the converting enzyme. *J Neurochem* 25:727-729, 1975
29. Uhlich E, Weber P, Elgler J, et al: Angiotensin stimulated AVP-release in humans. *Klin Wochenschr* 53:177-180, 1975
30. Andersson B, Larsson B: Influence of local temperature changes in the preoptic area and rostral hypothalamus on the regulation of food and water intake. *Acta Physiol Scand* 52:75-89, 1961
31. Grossman SP: Direct adrenergic and cholinergic stimulation of hypothalamic mechanisms. *Am J Physiol* 202:872-882, 1962
32. Levitt RA, Fisher AE: Anticholinergic blockade of centrally induced thirst. *Science* 154:520-521, 1966
33. Lehr D, Mallow J, Krukowski M: Copious drinking and simultaneous inhibition of urine flow elicited by beta adrenergic stimulation and contrary effect of alpha adrenergic stimulation. *J Pharmacol Exp Ther* 158:150-163, 1967
34. Leksell LG: Influence of PGE on cerebral mechanisms involved in control of fluid balance. *Acta Physiol Scand* 98:85, 1976
35. Andersson B, Leksell LG: Effects on fluid balance of intraventricular infusions of prostaglandin E₁. *Acta Physiol Scand* 93:286, 1975
36. Yamamoto M, Share L, Shade RE: Vasopressin release during ventriculocisternal perfusion with prostaglandin E₂. *J Endocrinol* 71:325, 1976
37. Fitzsimons JT, Setler PE: Catecholaminergic mechanisms in angiotensin induced drinking. *J Physiol* 218:43P-44P, 1971
38. DeRubertis FR, Michels MF, Beck N, et al: "Essential" hypernatremia due to ineffective osmotic and intact volume regulation of vasopressin secretion. *J Clin Invest* 50:97-111, 1971
39. Berl T, Anderson RG, Aisenbray GA, et al: On the mechanisms of the polyuria in potassium depletion: The role of polydipsia. *Clin Res* 25:136A, 1977
40. Schrub JCL, Dubuisson M, Hillermand B, et al: L'hypernatremie (Neurogenic hypernatremia)—A case noted during development of a pinealoma. *Sem Hop Paris* 46:2084-2089, 1970
41. Sawyer WH: Active neurohypophyseal principles from a cyclostome and two cartilaginous fishes. *Am J Med* 42:678-686, 1967
42. Sawyer WH: Evolution of neurohypophyseal hormones and their receptors. *Fed Proc* 36:1842-1847, 1977
43. Pavel S, Goldstein R, Calb M: Vasotocin content in the pineal gland of foetal, newborn and adult male rats. *J Endocrinol* 66:283-284, 1975
44. Rosenbloom AA, Fisher DA: Radioimmunoassayable AVT and AVP in adult mammalian brain tissue: Comparison of normal and Brattleboro rats. *Neuroendocrinology* 17:354-361, 1975
45. Skowsky WR, Fisher DA: Fetal neurohypophyseal arginine vasopressin and arginine vasotocin in man and sheep. *Pediatr Res* 11:627-630, May 1977
46. Vaughn MK, Blask DE, Vaughn GM, et al: Dose dependent prolactin releasing activity of arginine vasotocin in intact and pinealectomized estrogen-progesterone treated adult male rats. *Endocrinology* 99:1319, 1976
47. Chessman DW, Osland RB, Forsham PH: Suppression of the preovulatory surge of luteinizing hormone and subsequent ovulation in the rat by arginine vasotocin. *Endocrinology* 101:1194-1202, 1977
48. Peck JW, Blass EM: Localization of thirst and antidiuretic osmoreceptors by intracranial injections in rats. *Am J Physiol* 228:1501-1509, 1975
49. Van Gemert M, Miller M, Carey RJ, et al: Polyuria and impaired ADH release following medial preoptic lesioning in the rat. *Am J Physiol* 228:1293-1297, 1975
50. Rosenfeld M: The native hormones of the posterior pituitary glands: The pressor and oxytocic principles. *Bull Johns Hopkins Hosp* 66:398-403, 1940
51. van Dyke HB, Chow BF, Greep RO, et al: The isolation of a protein from pars neuralis of the ox pituitary with constant oxytocic, pressor, and diuresis inhibiting activities. *J Pharmacol Exp Ther* 74:190-209, 1942
52. Acher R, Chauvet J, Olivry G: Sur l'existence eventuelle d'une hormone unique neurohypophysaire—I. Relation entre l'ocytocine, la vasopressine et la proteine de van Dyke extraites de la neurohypophyse du boeuf. *Biochim Biophys Acta* 22:421-427, 1956
53. Acher R, Fromageot C: The relationship of oxytocin and vasopressin to active proteins of posterior pituitary origin—Studies concerning the existence or non-existence of a single neurohypophysial hormone. In Heller H (Ed): *The Neurohypophysis*. London, Butterworth and Company, Ltd, 1957, pp 34-37
54. Ginsburg M, Ireland M: The role of neurophysin in the transport and release of neurohypophysial hormones. *J Endocrinol* 35:289-298, 1966
55. Dean CR, Hope DB: The isolation of neurophysin I and II from bovine pituitary neurosecretory granules separated on a large scale from other subcellular organelles. *Biochem J* 106:565-573, 1968
56. Buford GD, Jones CW, Pickering BT: Tentative identification of a vasopressin-neurophysin and an oxytocin-neurophysin in the rat. *Biochem J* 124:809-813, 1971
57. Cheng KW, Friesen HG: Isolation and characterization of a third component of porcine neurophysin. *J Biol Chem* 246:7656-7665, 1971
58. Hollenberg MD, Hope DB: The isolation of the native hormone binding proteins from bovine posterior pituitary lobes. *Biochem J* 106:557-564, 1968
59. Cheng KW, Friesen HG: The isolation and characterization of human neurophysin. *J Clin Endocrinol Metab* 34:165-176, 1972
60. Ranch R, Hollenberg MD, Hope DB: Isolation of a third bovine neurophysin. *Biochem J* 115:476-479, 1969
61. Robinson AG, Zimmerman EA, Frantz AG: Physiologic investigation of posterior pituitary binding—Proteins neurophysin I and neurophysin II. *Metabolism* 20:1148-1155, 1971
62. Robinson AG, Zimmerman EA, Engleman EG, et al: Radioimmunoassay of bovine neurophysin: Specificity of neurophysin I and neurophysin II. *Metabolism* 20:1138-1147, 1971
63. Sachs H, Fawcett CP, Takabatake Y, et al: Biosynthesis and release of vasopressin and neurophysin. *Rec Progr Horm Res* 24:447-491, 1969
64. Sachs H, Goodman R, Osinchak J, et al: Supraoptic neurosecretory neurons of the guinea pig in organ culture—Biosynthesis of vasopressin and neurophysin. *Proc Natl Acad Sci USA* 68:2782-2786, 1971
65. Friesen HG, Astwood EB: Changes in neurohypophysial proteins induced by dehydration and ingestion of saline. *Endocrinology* 80:278-287, 1967
66. Fawcett CP, Powell AE, Sachs H: Biosynthesis and release of neurophysin. *Endocrinology* 83:1299-1310, 1968
67. Cheng KW, Friesen HG: Physiological factors regulating secretion of neurophysin. *Metabolism* 19:876-890, 1970
68. Cheng KW, Friesen HG: A radioimmunoassay for vasopressin binding protein, neurophysin. *Endocrinology* 88:608-619, 1971
69. Legros JJ, Frachimont P: The radioimmunoassay of bovine neurophysin: Evidence of a cross-reaction with a substance in human plasma. In Kirkham KE, Hunter WM (Eds): *Radioimmunoassay Methods*. Edinburgh, Churchill Livingstone, 1971, p 98
70. Sachs H, Portanova R, Haller EW, et al: Cellular processes concerned with vasopressin biosynthesis, storage, and release. In Stutinsky F (Ed): *Neurosecretion—Fourth International Symposium on Neurosecretion*. Berlin, Springer-Verlag OHG, 1967, p 146
71. Sachs H: Biosynthesis and release of vasopressin. *Am J Med* 42:687-700, 1967
72. Valtin H: Hereditary hypothalamic diabetes insipidus in rats (Brattleboro strain)—A useful experimental model. *Am J Med* 42:814-827, 1967
73. Cross BA, Dyball REJ, Dyer RG, et al: Endocrine neurons. *Rec Progr Horm Res* 31:243-294, 1975
74. Hayward JN: Neural control of the posterior pituitary. *Ann Rev Physiol* 37:191-210, 1975
75. Arnauld E, Vincent JD, Dreifus JJ: Firing patterns of hypothalamic supraoptic neurons during water deprivation in the monkey. *Science* 185:535-537, 1974
76. Wakerley JB, Poulain DA, Dyball REJ, et al: Activity of phasic neurosecretory cells during hemorrhage. *Nature* 258:82-84, 1975

CLINICAL PHYSIOLOGY OF WATER METABOLISM—PART I

77. Dreifus JJ, Harris ME, Tribollet E: Excitation of physically firing hypothalamic supraoptic neurons by carotid occlusion in rats. *J Physiol* 257:337-354, 1976
78. Zimmerman EA, Robinson AG: Hypothalamic neurons secreting vasopressin and neurophysin. *Kidney Int* 10:12-24, 1976
79. Zimmerman EA: Localization of hypothalamic hormones by immunocytochemical techniques. In Martini L, Ganong WF (Eds): *Frontiers in Neuroendocrinology*, Vol 4. New York, Raven Press, 1976
80. Zimmerman EA, Carmel PW, Husain MK, et al: Vasopressin and neurophysins—High concentrations in monkey hypophyseal portal blood. *Science* 182:925-927, 1973
81. Seif SM, Huellmantel AB, Shullman M, et al: Neurophysin and vasopressin in the plasma and hypothalamus of adrenalectomized and normal rats. *Endocrinology* 96:186A, 1975
82. Vorherr H, Bradbury MWB, Hoghoughi M, et al: Antidiuretic hormone in cerebrospinal fluid during endogenous and exogenous changes in its blood level. *Endocrinology* 33:246, 1968
83. Zaid SM, Heller H: Can neurohypophyseal hormones cross the blood-cerebrospinal fluid barrier? *J Endocrinol* 60:195-196, 1974
84. Van Wimersma Greidanus TB, Dogterom J, de Wied D: Intraventricular administration of antivasopressin serum inhibits memory consolidation in rats. *Life Sci* 16:637-644, 1975
85. Douglas WW, Nagasawa J, Schultz R: Electron microscopic studies on the mechanism of secretion of posterior pituitary hormones and significance of microvesicles ("synaptic vesicles"): Evidence of secretion by exocytosis and formation of microvesicles as a by-product of this process. In Heller H, Lederis K (Eds): *Memoirs of the Society for Endocrinology—Subcellular Organization and Function in Endocrine Tissues*. London, Cambridge University Press, 1971, pp 353-378
86. Nagasawa J, Douglas WW, Schultz RA: Ultrastructural evidence of secretion by exocytosis and of "synaptic vesicle" formation in posterior pituitary gland. *Nature* 227:407-409, 1970
87. Nagasawa J, Douglas WW, Schultz RA: Micropinocytotic origin of coated and smooth microvesicles ("synaptic vesicles") in neurosecretory terminals of posterior pituitary glands demonstrated by incorporation of horseradish peroxidase. *Nature* 232:341-342, 1971
88. Douglas WW, Nagasawa J, Schultz RA: Coated microvesicles in neurosecretory terminals of posterior pituitary glands shed their coats to become smooth "synaptic" vesicles. *Nature* 232:340-341, 1971
89. Barer R, Heller H, Lederis K: The isolation, identification and properties of the hormonal granules of the neurohypophysis. *Proc R Soc Lond (Biol)* 158:388-416, 1963
90. Gerschenfeld HM, Tramezzani JH, DeRobertis E: Ultrastructure and function in the neurohypophysis of the toad. *Endocrinology* 66:741-762, 1960
91. Paley S: The fine structure of the neurohypophysis. In Walsch H (Ed): *Ultra-Structure and Cellular Chemistry of Neural Tissue*, Progress in Neurobiology, Vol 2. New York, Paul B. Hoeber, Inc, 1957, pp 31-49
92. Douglas WW, Poisner AM: Stimulus-secretion coupling in a neurosecretory organ—The role of calcium in the release of vasopressin from the neurohypophysis. *J Physiol (London)* 172:1-18, 1964
93. Sachs H, Share L, Osinchak J, et al: Capacity of the neurohypophysis to release vasopressin. *Endocrinology* 81:755-770, 1967
94. Czaczkes JW, Kleeman CR: The effects of various states of hydration and the plasma concentration on the turnover of antidiuretic hormone in mammals. *J Clin Invest* 43:1625-1640, 1964
95. Miller L, Fisch L, Kleeman CR: Relative potency of arginine-8-vasopressin and lysine-8-vasopressin in humans. *J Lab Clin Med* 69:270-291, 1967
96. Lauson H: Metabolism of antidiuretic hormones. *Am J Med* 42:713-744, 1967
97. Sawyer WH: Biological assays for neurohypophysis principles in tissues and in blood. In Harris GW, Donovan T (Eds): *The Pituitary Gland*, Vol 3. Berkeley, The University of California Press, 1966, pp 288-306
98. Thomas TH, Lee MR: The specificity of antisera for the radioimmunoassay of arginine vasopressin in human plasma and urine during water loading and dehydration. *Clin Sci Molec Med* 51:525-536, 1976
99. Miller M, Moses AM: Radioimmunoassay of urinary antidiuretic hormone with application to study of the Brattleboro rat. *Endocrinology* 88:1389-1396, 1971
100. Moses AM, Streeten DHP, Streeten MD: Differentiation of polyuric states by measurement of responses to changes in plasma osmolality induced by hypertonic saline infusions. *Am J Med* 42:368-377, 1967
101. Moses AM, Miller M, Streeten DHP: Quantitative influence of blood volume expansion on the osmotic threshold for vasopressin release. *J Clin Endocrinol* 27:655-662, 1967
102. Verney EB: The antidiuretic hormone and the factors which determine its release. *Proc R Soc Lond (Biol)* 135:25-106, 1947-1948
103. Robertson GL, Mahr EA, Athar S, et al: Development and clinical application of a new method for the radioimmunoassay of arginine vasopressin in human plasma. *J Clin Invest* 52:2340-2352, 1973
104. Dunn FL, Brennan TJ, Nelson AE, et al: The role of blood osmolality and volume in regulating vasopressin secretion in the rat. *J Clin Invest* 52:3212-3219, 1973
105. Weitzman RE, Fisher DA: Log linear relationship between plasma arginine vasopressin and plasma osmolality. *Am J Physiol* 233:E37-E40, 1977
106. Eriksson L, Fernandez O, Olsson K: Differences in the antidiuretic response to intracarotid infusions of hypertonic solutions in the conscious goat. *Acta Physiol Scand* 83:554-562, 1971
107. Andersson B, Eriksson L: Conjoint action of sodium and angiotensin on brain mechanisms controlling water and salt balances. *Acta Physiol Scand* 81:18-29, 1971
108. Eriksson L: Effect of lowered CSF sodium concentration on the central control of fluid balances. *Acta Physiol Scand* 91:61-68, 1974
109. Robertson GL, Athar S: The interaction of blood osmolality and blood volume in regulating plasma vasopressin in man. *J Clin Endocrinol Metab* 42:613-620, 1976
110. Shimizu K, Share L, Claybaugh JR: Potentiation by angiotensin II of the vasopressin response to an increased plasma osmolality. *Endocrinology* 93:42-50, 1973
111. Gauer OH, Henry JP: Circulating basis of fluid volume control. *Physiol Rev* 43:423-481, 1963
112. Share L: Role of peripheral receptors in the increased release of vasopressin in response to hemorrhage. *Endocrinology* 81:1140-1146, 1967
113. Travis RH, Share L: Vasopressin-renin-cortisol interrelations. *Endocrinology* 89:246-253, 1971
114. Davies R, Slater JDH, Forsling ML, et al: The response of arginine and plasma renin to postural change in normal man with observations on syncope. *Clin Sci Molec Med* 51:267-274, 1976
115. Epstein M: Cardiovascular and renal effects of head out water immersion in man. *Circ Res* 39:619-628, 1976
116. Borst JGG, deVries LA: The three types of "natural" diuresis. *Lancet* 2:1-6, 1950
117. Cirksema WJ, Dirks JH, Berliner RW: Effect of thoracic cava obstruction in response of proximal tubule sodium reabsorption to saline infusion. *J Clin Invest* 45:179-186, 1966
118. Rector FC Jr, Sellman JC, Martinez-Maldonado M, et al: The mechanisms of suppressions of proximal tubular reabsorption by saline infusions. *J Clin Invest* 46:47-56, 1967
119. McCance RA: Experimental sodium chloride deficiency in man. *Proc R Soc Lond (Biol)* 119:245-268, 1936
120. Leaf A, Mamby AR: An antidiuretic mechanism not regulated by extracellular fluid. *J Clin Invest* 31:60-71, 1952
121. Leaf A, Frazier HS: Some recent studies on the actions of neurohypophyseal hormones. *Progr Cardiovasc Dis* 4:47-64, 1961
122. Weitzman R, Farnsworth L, MacPhee R, et al: The effect of opposing osmolar and volume stimuli on plasma arginine vasopressin (pAVP) in man. *Mineral Elec Metab* 1:43-47, 1978
123. Barker JL, Crayton JW, Nicoll RA: Noradrenaline and acetylcholine response of supraoptic neurosecretory cells. *J Physiol* 218:19-32, 1971
124. Berl T, Cadnaphornchai P, Harbottle JA, et al: Mechanisms of suppression of vasopressin during alpha adrenergic stimulation with norepinephrine. *J Clin Invest* 53:219-227, 1974
125. Shimamoto K, Miyahara M: Effect of norepinephrine infusion on plasma vasopressin levels in normal human subjects. *J Clin Endocrinol Metab* 43:201-204, 1976
126. Schrier RW, Lieberman R, Ufferman RC: Mechanism of antidiuretic effect of beta-adrenergic stimulation. *J Clin Invest* 51:97-111, 1972
127. Klein LA, Liberman B, Laks M, et al: Interrelated effects of antidiuretic hormone and adrenergic drugs on water metabolism. *Am J Physiol* 221:1657-1665, 1971
128. Levi J, Grinblat J, Kleeman CR: Effect of isoproterenol on water diuresis in rats with congenital diabetes insipidus. *Am J Physiol* 221:1728-1732, 1971
129. Gill JR Jr, Casper ACT: Depression of proximal sodium reabsorption in the dog in response to renal beta-adrenergic stimulation by isoproterenol. *J Clin Invest* 50:112-118, 1971
130. Fisher DA: Norepinephrine inhibition of vasopressin antidiuresis. *J Clin Invest* 47:540-547, 1968
131. Baldes EJ, Smirk FH: The effect of water drinking, mineral starvation and salt administration on the total osmotic pressure of the blood in man, chiefly in relation to the problems of water absorption and water diuresis. *J Physiol* 82:62-74, 1934
132. Wood P: Polyuria in paroxysmal tachycardia and paroxysmal atrial flutter and fibrillation. *Br Heart J* 25:273-282, 1963
133. Fichman MP, Vorherr H, Kleeman CR, et al: Diuretic-induced hyponatremia. *Ann Intern Med* 75:853-863, 1971
134. Skowsky WR, Rosenbloom AA, Fisher DA: Radioimmunoassay measurement of arginine vasopressin in serum: Development and application. *J Clin Endocrinol Metab* 38:278-287, 1974
135. Beardwell CG, Geelen G, Palmer HM, et al: Radioimmunoassay of plasma vasopressin in physiological and pathological states in man. *J Endocrinol* 67:189-202, 1975
136. Morton JJ, Padfield L, Forsling ML: A radioimmunoassay for plasma arginine vasopressin in man and dog—Application to physiological and pathological states. *J Endocrinol* 65:411-424, 1975
137. Rabkin R, Young J, Crofton J, et al: The renal handling of arginine vasopressin. *Clin Res* 25:62A, 1977
138. Levi J, Rosenfeld S, Kleeman CR: Inactivation of arginine vasopressin by the isolated perfused rabbit kidney. *J Endocrinol* 62:1-10, 1974
139. Weitzman RE, Fisher DA: Arginine vasopressin metabolism in dogs—I: Evidence for a receptor-mediated mechanism. *Am J Physiol* 235:E591-E597, Dec 1978
140. Willson KC, Weitzman RE, Fisher DA: Arginine vasopressin metabolism in dogs—II: Modeling and system analysis. *Am J Physiol* 235:E598-E605, Dec 1978
141. Robertson GL, Mahr EA, Athar S, et al: Development and clinical application of a new method for the radioimmunoassay of arginine vasopressin in human plasma. *J Clin Invest* 52:2340-2352, 1973
142. Weitzman RE, Fisher DA, Di Stefano JJ IV, et al: Episodic secretion of AVP. *Am J Physiol* 233:E32-E36, 1977